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Abstract

Introduction

Lifestyle factors, such as diet, physical activity and sleep, are associated with the development of many chronic diseases. The objective of The Manitoba Personalized Lifestyle Research (TMPLR) study is to understand how these lifestyle factors interact with each other and additional factors, such as an individual's genetics and gut microbiome, to influence health.

Methods

An observational study of adults, with deep phenotyping by objective health and lifestyle assessments, multi-omic analyses, and retrospective assessment of early life experiences, with retrospective and prospective utilization of secondary data from administrative health records.

Study population

A planned non-random convenience sample of 840 Manitobans aged 30-46 recruited from the general population, stratified by sex (equal males and females), body mass index (BMI; 60% of participants with a BMI >25), and geography (25% from rural areas, accessed using a mobile research unit).

Measurements

Lifestyle factors assessed will include dietary pattern, physical activity, cardiovascular fitness and sleep. Additional factors such as medical history, socio-economic status, alcohol and tobacco consumption, cognition, stress and anxiety, and early life experiences will also be documented. A maternal survey will be performed. Body composition and bone density will be measured by dual energy x-ray absorptiometry. Blood pressure, pulse wave velocity, and augmentation index will be measured on two consecutive days. Chronic disease risk biomarkers will be measured in blood and urine samples. DNA will be extracted for genetic analysis. A fecal sample will be collected for microbiome analysis. Participants may provide their Manitoba Personal Health Information Number (PHIN) to link their study data with administrative health records.

Ethics and dissemination

Ethics approval has been obtained from the University of Manitoba Health Research Ethics Board (protocol # HS18951; 05/01/2016). Data analysis, release of results, and publication of manuscripts are scheduled to start in late 2018. Additional information at www.TMPLR.ca.

Clinicaltrials.gov NCT#xxxxxx

Article Summary

Strengths and limitations of this study

The study is designed to capture deep phenotyping of participants in the areas of diet, physical activity and sleep, combined with genetic and gut microbiome profiles. The ability to link these study data to healthcare usage data retrospectively and prospectively is a key strength of the research (**Figure 1**). The use of a mobile research unit to access rural populations makes the study unique as geographic setting can strongly influence health-related behaviors.

The study uses non-random convenience sampling for feasibility reasons, which can introduce selection bias and limit generalizability; however, preset stratification based on sex, BMI, and geography have been implemented to enhance representation. Another limitation is that some of the questionnaires used in TMPLR have not previously been validated, or not validated in the specific TMPLR study population. Finally, the study sample size of 840 individuals was not selected to power a specific primary hypothesis; however, it will provide a one-of-a-kind research platform of deeply phenotyped participants in which to investigate the associations between lifestyle and chronic disease.

Introduction

Approximately half of Manitobans are living with at least one of the following chronic conditions: obesity, hypertension, type 2 diabetes (T2D), cardiovascular disease (CVD), or chronic kidney disease (CKD) [2]. The consiquences of these chronic conditions is substantial and the financial burden, both personally and societally, is enormous. In the province of Manitoba, which has a universal healthcare system and a population of approximately 1.2 million, over 40 percent of total provincial revenues are spent on healthcare[1]. The burden of conditions like T2D and CKD are not unique to Manitoba [2, 3], therefore the primary and secondary prevention of these chronic conditions is a major international health research priority [4] .

It is well established that diet, physical activity, and sleep influence health and mortality [5-8]. Evidence-based guidelines pertaining to nutrition, physical activity and sleep exist to educate the public on healthy lifestyle choices. However, most current lifestyle guidelines follow a one-size-fits-all format, even though they are intended for populations comprising individuals with diverse and complex health circumstances and unique factors influencing their ability to follow the guidelines. This format may be a contributing factor to the poor adherence to lifestyle guidelines. For example, although most people are aware that physical activity is important for health, only 15% of the Canadian population achieve the national recommendations [9]. Similarly, it is estimated that 50% of women and 70% of men in Canada have energy intakes that exceed their energy needs, while 50% to 90% have deficiencies in calcium and vitamin D [10].

There is now an increasing interest in the creation of lifestyle strategies or guidelines for specific sub-populations or groups of individuals with specific characteristics [11-13]. It is hoped that such tailored recommendations will be more effective, and that barriers to healthy lifestyle practices can be ameliorated through personalization. Current one-size-fits-all recommendations and strategies may not be effective due to (1) significant inter-individual variability or (2)shared circumstances, such as geography, sleep/wake pattern, or socio-economic status, of a particular group.

We hypothesize that an individual's lifestyle will be influenced by socio-economic status and geography, and will interact with their genotype and gut microbiota to affect health [14, 15]. Accordingly, The Manitoba Personalized Lifestyle Research (TMPLR) study will involve the coordinated collection of data related to socio-economic status, geography, nutrition, physical activity, sleep, early life experiences, and health systems usage, in conjunction with the analysis of genetics, gut microbiota, and risk factors for chronic conditions such as obesity, hypertension, T2D, CVD, and CKD. After establishing the baseline characteristics of this study cohort, administrative health records will be used retrospectively to examine the developmental origins of health and disease, and prospectively to track and investigate the development of chronic disease in the future. Consent has been obtained to contact study participants for further clinical assessments, contingent on future funding.

Data from this study will provide an ideal opportunity for the discovery of new interactive mechanisms through which lifestyle factors affect health. These interactive mechanisms may also address the question of why some people are more successful than others in changing their lifestyle as it relates to chronic conditions. Findings from this research will be useful in guiding both clinical and health policy decisions, and will also facilitate the design and testing of personalized health promotion strategies.

Methods

Design

This is an observational cohort study with retrospective and prospective utilization of secondary data from administrative health records (**Figure 1**). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed where applicable in the development of this protocol manuscript [16].

Setting

Urban (Winnipeg) and rural (Morden, Winkler, Carman, Steinbach) areas with road access in southern Manitoba, Canada.

Objectives of the study

The objective of this study is to explore the complex interactions that exist between lifestyle, genetics, and gut microbiota, and how these relate to risk factors for chronic conditions, especially obesity, hypertension T2D, CVD, and CKD in Manitoba.

Inclusion and exclusion criteria

A sample of 800 Manitobans aged 30-46, stratified by sex, BMI, and geography **(Table 1)** are being recruited. Participants must have lived in Manitoba for a minimum of 5 years. Women who are pregnant or lactating are not eligible to participate. Additionally, because it is expected that very few of the 800 Manitobans who join TMPLR study from the general public will have reduced kidney function (eGFR <30 ml/min), 40 participants (20 female, 20 male, with no set stratification based on BMI or geography) who have severely reduced kidney function are being recruited from the renal health clinic at Seven Oaks General Hospital (SOGH), Winnipeg, MB. Therefore, the study has a recruitment goal of 840 participants.

Recruitment

Participants are recruited through the use of printed flyers, online advertisements purchased via Google, Facebook, and Twitter ad platforms and social media accounts, appearances in local TV, radio, and print media, and direct contact with community groups, such as churches, sports leagues, and community clubs. All patients who receive care in the SOGH renal health clinic, who are aged 30-46, have been living in Manitoba for a minimum of the last 5 years, and are able to provide informed consent are approached to enroll in the study as well.

Sample size

The sample size of TMPLR study was selected based on considerations of feasibility of recruitment, costs, and logistics. However, given established values from other sources [17] and our anticipated sample size of 840 participants, we estimate that will have we will have 80% power (5% significance, 2-sided) to detect a minimum body fat difference of 2.5% for rare exposures (i.e., experienced by 10% of participants, such as smoking) and 1.7% for more common exposures (experienced by 25% of participants, such meeting the Canadian recommended 150 minutes of moderate-to-vigorous physical activity). Additional estimated minimum detectable differences are presented in **Table 2**. These lower limits should allow for the detection of clinically meaningful changes in these outcomes.

Data Collection and Assessments

On two consecutive days, participants come to either the urban TMPLR study site at the Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba or TMPLR's mobile research unit which travels to other areas of Winnipeg and southern Manitoba. TMPLR's mobile research unit is a custom built 12-meter mobile lab which is equipped with phlebotomy area, a dual-energy X-ray absorptiometer (DXA) and a bicycle ergometer with a metabolic cart. During this visit, participants complete questionnaires, undergo various health assessments, provide urine and fecal samples, and have fasting blood samples taken **(Figure 2, Table 3)**.

Questionnaires

Questionnaires capture socio-demographic characteristics, personal and family medical history, smoking (including electronic cigarette use), current diet (three Automated Self-Administered 24-h (ASA24) Dietary Assessment Tool recalls [18] , Mindful Eating Questionnaire [19], Diet History Questionnaire [20] and The Three-Factor Eating Questionnaire [21]), alcohol consumption, physical activities, frailty using the Modified Fried Criteria [22], stress, sleep (Pittsburgh Sleep Quality Index [23]), cognition (Montreal Cognitive Assessment Questionnaire [24]), and childhood retrospective circumstances (adapted from the US Panel Study on Income Dynamics [25]).

Anthropometric assessment

Weight is measured after participants change into lightweight scrub tops and bottoms, with shoes removed, to the nearest 0.1 kg using a digital calibrated floor scale (7562EF, Taylor Precision Products, Oak Brook, IL, USA). Height is measured, without shoes, to the nearest 0.1 cm using a stadiometer (Model 206, SECA North America, Chino, CA, USA). BMI is calculated in kg/m^2. Waist circumference is measured in triplicate, to the nearest 0.1 cm at the umbilicus, between the last rib and the iliac crest using a fibreglass tape measure. Hip circumference is measured in triplicate at the widest portion of the buttocks and hips using a fibreglass tape measure. Body composition including fat mass, lean mass, percent body fat, visceral adipose tissue (VAT), and bone mineral density (BMD) are assessed using dual-energy X-ray absorptiometry (DXA, Lunar Prodigy Advance, GE Healthcare, Mississauga, ON, CAN) [26]. Scans are taken of the whole body, femoral neck, L1-L4 of the spine, and the non-dominant forearm.

Clinical health assessment

Participants' systolic and diastolic blood pressures are measured in triplicate, on the non-dominant arm in a sitting position using a validated oscillometric blood pressure monitor (BP760CAN, Omron, Burlington, ON, CAN). Participants are required to rest for 5-10 minutes before taking the measurement. Pulse wave velocity and augmentation index are measured on the non-dominant arm in a sitting position using a Mobil-O-Graph PWA Monitor and the HMS Client Server Software (I.E.M Gmbh, Stolberg, Germany) according to the manufacturer's protocol on two consecutive days [27].

Collection of bio-specimens

Blood, urine, and fecal samples are obtained from study participants **(Supplementary protocols)**. Fasting blood samples are collected on two consecutive days via venipuncture by trained phlebotomists. Participants are asked to collect two urine samples at home; one sample is obtained prior to going to bed, and a second of the first morning void upon waking up.

Participants also collect a fecal sample; they are provided a collection kit and instructed to collect a single sample from 3 separate places on the stool using a spoon attached to the cap of the collection tube. Participants are instructed to store the collected samples fecal in their household –20°C freezer with a provided ice pack, and Urine samples in the fridge, until transport back to the study center, using provided ice pack for temperature control, where they are aliquoted and then stored at –80°C for future analysis [28].

Clinical chemistry in blood and urine

Clinical chemistry, including lipid profile, glucose, insulin, and renal and liver profiles will be measured via automated clinical chemistry analyzers (Cobas C111, C311 and e411, Roche Diagnostics Laval, QC). Additional blood and urine biomarkers such as leptin, glucagon, and melatonin will be measured via ligand binding assay (LBA) or enzyme-linked immunosorbent assay (ELISA). Red blood cell and plasma fatty acids will be measured by gas chromatography with flame ionization detections (GC-FID)[29]. Non-cholesterol sterols will be measured in plasma using GC-FID and mass spectrometry (MS) [30]. Vitamin C concentrations in the blood will be measure by high pressure liquid chromatography (HPLC) [31].

Microbiome analyses in fecal samples

Fecal samples will be subjected to genomic DNA extraction (Zymo Research, CA, USA) following the manufacturer's protocol. Experimental negative controls will be included in extraction protocols to confirm the reliability and consistency of the extracted nucleic acid. The V4 hypervariable region of 16S rRNA gene will be amplified, the sequencing library will be generated as described previously [32] and sequenced at the Gut Microbiome Laboratory, University of Manitoba. Samples will be multiplexed at the rate of 200 per run aiming for an average sequencing depth of 50,000 sequences per sample. The sequencing data will be deposited into the Sequence Read Archive (SRA) of NCBI (http://www.ncbi.nlm.nih.gov/sra) and accession numbers will be provided for future access.

Deuterium oxide administration

After the blood sample collection on day one, participants are given 0.7 grams of deuterium oxide per kilogram of estimated body water to drink. Body water is estimated as body weight (kg) x 0.60. This deuterium administration is used to enrich the body's water pool for the assessment of fractional cholesterol and triglyceride synthesis rates [33-35].

Physical activity and capacity testing

Physical activity level in TMPLR study participants is assessed using accelerometers (Actigraph GTX3bt, Penscola. FL, USA) worn for 1 week [36, 37]. Muscle strength is measured using a hand grip dynamometer. Cardiorespiratory fitness is assessed using a submaximal bike protocol which includes heart rate monitoring, and a metabolic cart (VMAX Encore, Carefusion, Unionville, Ont., Canada) to measure oxygen consumption and CO_2 output. Functional walking ability is assessed using a 5-meter gait speed test. Additionally, depressive symptoms, obesity history, frailty, low physical activity, and cognitive impairment are assessed by validated questionnaires [38-40].

Sleep assessment

Sleep in TMPLR study participants is measured objectively using accelerometers (Actigraph GTX3bt, Penscola. FL, USA [41]) worn for a week and subjectively by questionnaire (Pittsburgh Sleep Quality Index [23]). While there is a strong relationship between objective and subjective sleep reports, TMPLR study is collecting both because discrepancies may provide important clinical information reflecting early dysfunction [42, 43].

Dietary assessment

Study participants complete the Canadian version of the Diet History Questionnaire (DHQ), which estimates the intake of common food items and includes portion size and dietary supplement questions. This questionnaire is on a TELEform for scanning data entry and creation of the data files. Participants also complete three dietary recall surveys using the Automated Self-Administered 24-hour Canada (ASA24®, NCI, Rockville, Maryland U.S. http://asa24.ca/) dietary assessment tool, a web-based tool that enables multiple, automatically coded, self-administered 24-hour recalls. Participants enrolled from March 2016 to February 2017 used the ASA24-Canada-2014 edition; those enrolled after February 2017 used the ASA24-Canada-2016 edition. Both ASA24-Canada-2014 and ASA24-Canada-2016 use the same nutrient databases.

Early life experiences

Early-life exposures spanning the critical time windows of fetal development, birth, infancy and early childhood are documented in three ways: 1) through linkage with administrative health records (described below), 2) by self-report, and 3) by maternal report. Mothers of TMPLR study participants are asked to complete a TMPLR Mother's Questionnaire, adapted from the Nurses' Health Study [44], capturing key pregnancy, birth, and postpartum events such as: method of birth; gestational age and birth weight; socioeconomic status at birth; maternal pre-pregnancy BMI and gestational weight gain; maternal smoking and diabetes during pregnancy; maternal prenatal care; breastfeeding initiation, exclusivity and duration; stressful life events during pregnancy and postpartum; and severe illness requiring hospitalization during infancy or early childhood. Early childhood socioeconomic status [45, 46] and stressful life events [47, 48] are also self-reported by TMPLR participants using the Childhood Retrospective Circumstances Questionnaire, adapted from the US Panel Study of Income Dynamics [25].

Data quality assurance and control

Methods of data collection (questionnaires, anthropometric assessment, and clinical health assessment) were standardized across the urban and mobile TMPLR study sites. Training of TMPLR study staff involved in data collection and data entry is regularly refreshed and all staff handling participant data are trained in compliance with the Manitoba Personal Health Information Act (PHIA). All data are entered in the secure TMPLR study digital platform, CREDIT (described below).

Questionnaire data entry is conducted via two methods. Questionnaires collected between March 2016 to October 2017 were collected on paper and entered manually by study staff. Questionnaires collected from October 2017 onwards are entered directly by participants using a digital questionnaire platform designed for TMPLR study using Clinical Research Electronic Data InfrastrucTure (CREDIT) software (FunctionFour Inc., Winnipeg, MB, Canada), unless the participant requests paper data collection. The digital platform allows for questionnaires to be completed using a computer, tablet, or smartphone. Data fields have limits to check the logic and

reasonable range of responses. The manually entered data are checked for contradictory responses in the double entry and manually corrected.

Any access of, or corrections made to, TMPLR study data in the digital platform are logged. To protect the confidentiality of participants, all clinical data, questionnaire data and laboratory data are kept secure and are identified only be a unique identifier. Participants' names, date of births, addresses, and PHINs are kept in separately encrypted fields within the CREDIT software platform. All data within the digital platform are encrypted at rest, using an encryption key that is separate from the highly identifiable data listed above. Access to TMPLR study data and de-encryption keys is only available for authorized research staff via a data manager. The electronic data are stored on a server at the study site and are mirrored at a secure off-site location. A TMPLR study data model has been created to help in visualizing the different types of data. **(Supplementary figure 1)**

Linkage to administrative health data

At enrollment, TMPLR participants are asked to provide their PHIN and grant permission to link their study data with administrative health records (including hospital discharge abstracts, physician billing claims, and prescription records). These data are accessed through the Manitoba Centre for Health Policy (MCHP) Population Research Data Repository [49] and linkage is achieved using the PHIN, following the standard procedures established by the MCHP and the Manitoba Health Information Privacy Committee. The data linkage is used to capture retrospective information on early life as well as prospective information on numerous health outcomes, including diagnosis of hypertension, T2D, CVD, and CKD.

Statistical analyses

Statistical analyses will be undertaken in consultation with biostatisticians from the George and Fay Yee Centre for Healthcare Innovation (CHI) at the University of Manitoba. Lifestyle factors will primarily be used as explanatory variables, with chronic disease biomarkers or disease presence/absence as outcomes, in multivariable regression models. Moderating or mediating effects of genetics, gut microbiome, clinical characteristics, socio-economic status, and environmental factors will be explored. The potential confounding effects of health status and healthcare use on variable relationships will be examined using techniques such as propensity score or instrumental variable models [50-52].

Techniques appropriate for high-dimensional data will be adopted where needed. For example, clustering of lifestyle risk factors will be examined using latent variable modeling techniques (i.e., latent class analysis). Dimension reduction techniques for omics data, such as microbiome and genetic markers, will be applied [53].

The bioinformatics and statistical analyses of microbiome data will be performed as described previously [32] and will be updated based on recommendations and technology advancements between now and processing of samples. Overall microbiota community structures, alpha diversity metrics, and relative abundances of operational taxonomic units (OTUs), will be tested for associations with lifestyle and health measures, with appropriate adjustment for multiple comparisons.

Non-response bias may affect the validity of analyses for survey data, necessitating the use of multiple imputation methods if the pattern of missing data is deemed to be ignorable [54]. For non-ignorable missing data, selection and pattern mixture models will be examined in sensitivity analyses [55]. Due to the use of non-random sampling there is a risk of selection bias; survey weights and weighting of responses may be used to address this bias. Standardization or adjustment techniques may be used to address bio-specimen measurement error bias [56].

Specialized methodological investigations will be conducted for: 1) psychometric analyses of scales, including testing for differential item functioning and measurement invariance [57-59], 2) development of chronic disease risk prediction models [60, 61], 3) techniques to evaluate the quality of linked databases, including their accuracy, reliability, and completeness [62], 4) robust statistical methods for the analysis of outcome measures with non-normal (e.g. skewed) distributions [63, 64].

Public engagement

Three focus group, one for healthcare providers and two for general public, and a public forum were held in the early design stages of this study to get input from Manitobans, on the study design and recruitment strategies. A study advisory board was also formed, and meets on a bi-annual basis. This advisory board includes healthcare providers, health researchers, and members of the public. The board provides input regarding study recruitment, progress and conduct, and will also provide input and suggestions regarding the dissemination of study results.

Provision of clinical results to participants

Individual results of the anthropomorphic measurements, blood pressure, pulse wave velocity, augmentation index, body composition, bone density, full lipid profile, fasting blood glucose, and renal and liver profile are provided to participants. They are referred to their primary care providers for further management if their results are beyond clinical reference ranges.

Ethics and dissemination

Explicit informed consent is obtained from each individual prior to participation in the study. Eligible participants are verbally informed by trained research personnel regarding the nature and purpose of the study, given time to decide whether or not to participate, and have any questions or concerns answered prior to consent and at any point throughout the study. All participants are informed that they may withdraw from the study at any time without penalty and are reimbursed for the portion of the study that they have completed up to that point.

Ethics approval has been obtained from the University of Manitoba Health Research Ethics Board prior to participant recruitment (protocol# HS18951). The study protocol has also been reviewed and approved by the Manitoba Health Information Privacy Committee in regards to the collection and use of PHIN, The St. Boniface Hospital Research Review Committee in regards to the processing of samples at the hospital, and the Winnipeg Regional Health Authority (WRHA) Research Access and Approval Committee (RAAC), the Southern Health Research Ethics Board, and the Interlake-Eastern Regional Health Authority Regional Ethics Committee, in regards to the study taking place in those health regions.

Data analysis, release of results, and publication of initial manuscripts are scheduled for 2019. Findings will be shared in peer-reviewed journals, and at regional, national, and international

scientific conferences. Data and findings will also be presented to healthcare policymakers within Manitoba, to develop preventive strategies that reduce chronic conditions with the intention of reducing healthcare costs. Funding applications for future clinical follow in this study population will also be submitted starting in 2019**.**

Discussion

TMPLR study has been uniquely designed to provide cross-sectional, retrospective and prospective observations that will improve our understanding of how lifestyle factors interact with each other and additional factors such as genetics and the gut microbiome to influence health and the risk of obesity, T2D, CVD, and CKD**.** The coordinated collection of lifestyle-gene-environment-microbiota-health data including objective measurements such as DXA, activity monitoring, stable isotopic tracer methodologies, and direct measurement of physiological biomarkers, combined with the ability to retrospectively assess and prospectively follow health outcomes in participants using administrative health records, represents an unprecedented opportunity to collect data which can be used to improve chronic disease prevention and management.

Due to the voluntary non-random recruitment of participants, there may be an under-representation of those with lower health awareness, financial means, access, or time to participate. Attempts to counteract this are implicit in the stratified recruitment design. Comparisons between TMPLR study participants and general Manitoban population demographics may allow assessment of potential selection biases. A healthy volunteer effect may impact the ability to detect weak associations between lifestyle and disease risk, but this may attenuate with longer follow-up using administrative health data.

In summary, TMPLR study will provide a unique platform of deeply phenotyped individuals that will be used to explore the interactions between lifestyle factors that associate with the development of, or protection from, obesity, hypertension, T2D, CVD, and CKD. The findings from this research platform will subsequently be used to develop and test preventive and restorative lifestyle and health strategies with the aim of improving the health and reducing healthcare costs at the individual and population levels.

Study status

Data collection started in March 2016. As of the end of January 2018, data collection has been completed for approximately 650 participants. The data collection and blood sampling are expected to be completed by late 2018.

Author contributions

DSM and RCM developed the original concept of the study for the original grant application with input from co-investigators. DSM prepared the drafts of the study protocol manuscript and compiled feedback and changes from other authors. MG assisted in the preparation of the study protocol manuscript. PF developed the branding for TMPLR study, and the manuscript figures and tables. NM prepared the data model and was involved in the public engagement. SB (project lead, indigenous health), HB (project lead, nutrition), JC, TAD (project lead, physical activity), PKE (project lead, genetics), EK (project lead, gut microbiome), LML (project lead, biostatistics), DEM (project lead, sleep), SBM, AR, NT, MBA (co-director and project lead, developmental origins of chronic disease), and PJJ (senior-director) are study co-investigators,

and were all involved in writing the original grant application. All authors have carefully read, contributed to, and approved the final version of the study protocol manuscript.

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Competing interests statement

DSM, RCM, MG, SB, HB, JC, TAD, PKE, PF, NH, EK, LML, DEM, SBM, AR, MBA, and PJJ have no competing interests to declare.

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Figure 1. The Manitoba Personalized Lifestyle Research Study overview

Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule

Table 1. The Manitoba Personalize Lifestyle Research Study recruitment targets by strata

Age	30-46 years N=800							
Sex 50% Male 50% Female	400 Males				400 Females			
Geography 72% Urban 28% Rural	288 Urban males		112 Rural males		288 Urban females		112 Rural females	
BMI 40% Normal (BMI <25) 60% Overweight	116 Urban males BMI	172 Urban males BMI	45 Rural males BMI	67 Rural males BMI	116 Urban females BMI	172 Urban females BMI	45 Rural females BMI	67 Rural females BMI

(BMI ≥ 25)	<25	≥ 25	<25	≥ 25	<25	≥ 25	<25	≥ 25
+40 participants with severely reduced kidney function (eGFR <30 ml/min), 20 female, 20 male, with no set stratification based on BMI or geography								

Table 2. The Manitoba Personalized Lifestyle Research (TMPLR) study estimated minimum detectable differences

Variable	Mean or median used	Standard deviation used	Minimum difference at 10% exposure (percentage of mean)	Minimum difference at 25% exposure (percentage of mean)	References
Body fat (%)	41.3% Females 27.8% Males	7.7% 6.6%	2.5% (6.0)	1.7% (4.0)	[17]
Lumbar bone mineral density (BMD; g/cm^2)	1.042 Females 1.058 Males	0.121 0.127	0.041 (3.8)	0.028 (2.6)	[65]
Glomerular filtration rate (GRF; ml/min per 1.73 m^2)	107.6	16.8	5.4 (5.0)	3.8 (3.5)	[66]
Systolic blood pressure (mmHg)	116	12	6.5 (5.6)	4.5 (3.9)	[67]
Fasting Glucose (mmol/L)	4.94	0.61	0.20 (4.0)	0.14 (2.8)	[67]
Fasting insulin (µIU/mL)	7.83	7.50	2.40 (30)	1.67 (21%)	[68]

LDL cholesterol (mmol/L)	2.79	0.67	0.22 (7.8)	0.15 (5.4)	[67]
Waist circumference (cm)	80	10	3.2 (4)	2.2 (2.75)	[67]

Table 3. The Manitoba Personalized Lifestyle Research (TMPLR) study data, assessment tools and biological samples

Characteristic	***Data***	***Method, Instrument or Source***
Sociodemographic	Date of birth, Sex, Ethnicity, Marital status	TMPLR study questionnaire
Medical	Personal medical history, Family medical history, Medication(s), Pregnancy history	TMPLR study questionnaire, Administrative health records
	Cognition	Montreal Cognitive Assessment [24]
Lifestyle	Tobacco/smoking/vaping use, Alcohol use, Unintentional weight loss, Exhaustion, Depression	TMPLR study questionnaire
Physical activity	Frailty	Modified Fried Criteria [22]
	Physical activity	Paffenbarger physical activity index, Actigraphy [36, 37]
	Predicted VO2 max	Modified YMCA bike test with metabolic cart
Nutrition	Dietary patterns and habits	Mindful eating questionnaire[19], three-factor eating questionnaire [21], automated 24-hour dietary recall [18], Canadian dietary history questionnaire [20]
Early life	Childhood health, socio-demographic and socioeconomic status; Parental employment history	Childhood retrospective questionnaire, adapted from the US Panel Study on Income Dynamics[25]
	Maternal: pregnancy events, obstetrical history, infant feeding	TMPLR Mother's retrospective childhood questionnaire, adapted from the Nurses Health Study [44]
Socioeconomic	Employment, Home ownership, Education attainment, Income	TMPLR study questionnaire
Sleep and Stress	Duration of sleep	Actigraphy [41]
	Sleep Quality	Pittsburgh sleep quality index [23]
	Perception of stress, Daily life stressors	Community-based stress and coping survey
Anthropometric	Height	Wall-mounted stadiometer

	Weight	Digital scale
	Waist circumference, Hip circumference	Tape measure
	Body fat, Lean mass, bone mineral density	Dual energy X-ray absorptiometry [26]
Blood pressure	Systolic & diastolic	Automated sphygmomanometer
	Pulse wave velocity, Augmentation index	Mobil-O-Graph oscillometer [27]
Biomarkers	Blood clinical chemistry and biomarker assays	Fasting blood samples
	Urinary clinical chemistry and biomarker assays	Urine samples
	Microbiome 16S RNA sequencing	Fecal sample [32]

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664

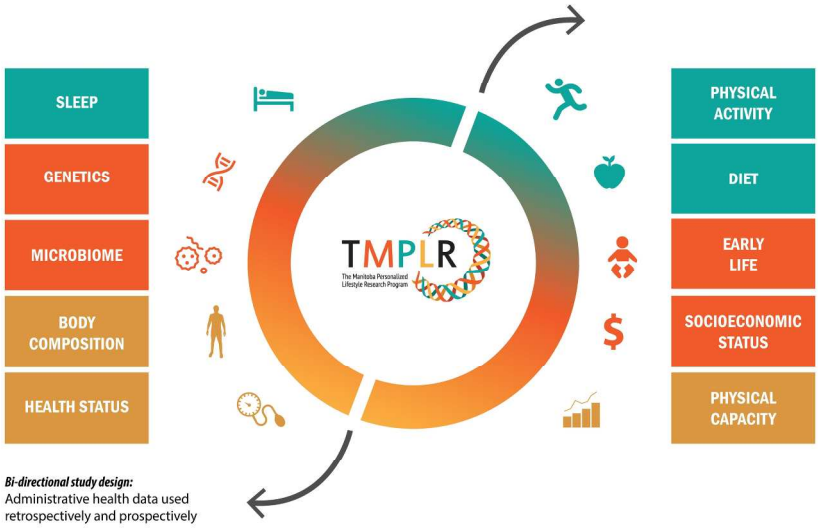

Figure 1. The Manitoba Personalized Lifestyle Research Study overview

230x130mm (300 x 300 DPI)

Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule

279x361mm (300 x 300 DPI)

TMPLR Data Model

Linking key: TMPLR ID #

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Urine Sample Collection Instructions

Please follow these instructions for urine collection. Research personnel will provide you with 2 urine collection cups labeled with time (night and day) and your TMPLR Study ID Number.

1. **Check your study ID on the collection tubes.** If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the samples.

2. Collect urine before going to bed tonight in the cup labeled "night". **Please write down the date and time of the sample was collected**. Store the sample in the fridge in the Ziploc bag provided.

3. Collect urine from the first time you pee after getting up in the morning, in the cup labeled "day". **Please write down the date and time of the sample was collected**. Store your samples in the fridge in the Ziploc bag provided.

4. Please bring the urine samples with you on your day 2 visit. TMPLR staff will collect the samples from you when you arrive.

If you have any questions, please contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.

Thank you for your cooperation!

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The Manitoba Personalized Lifestyle Research (TMPLR) Study

Stool Sample Collection Instructions

1. Freeze the ice packs provided by the study once you get home.

2. **Check your study ID** on the collection tubes (the two plastic tubes with blue cap). If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the sample.

3. Empty your bladder. Flush toilet. Place the collection unit under the rear part of the toilet seat with the round side pointing towards the back.

4. Have a bowel movement. **Collect 2 samples, one in each plastic tube, from 3 different places of the stool** using the spoon attached to the cap of the collection tubes. **Fill each sample tube about one third of the tube with stool sample**.

5. Close the tube tightly. Place each tube in a Ziploc bag provided. **Write down the date and time of the bowel movement** on the bag. Discard the used collection unit.

6. Wrap the collection tubes with the frozen ice packs, and keep them in the paper bag provided. Keep the collected sample in the freezer.

7. Return the stool samples wrapped with the frozen ice packs on day 2 of the measurements, or as soon as you can. TMPLR staff will collect the sample from you in the paper bag when you arrive.

If you have any questions, feel free to contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.

Collection Unit

Collection Tube with Spoon

Biospecimens collection

Blood samples will undergo analysis for numerous established and emerging health biomarkers, these include: total cholesterol, LDL-C, HDL-C, triglycerides, glucose, AST, ALT, insulin, glucagon-like peptide-1(GLP-1), leptin, c-reactive protein (CRP), fatty acids, HbA1c, T-regs, serum creatinine, blood urea nitrogen (BUN), non-cholesterol sterols, adipokines, cytokines,vitamin C, fat soluble vitamins, and lipidomic and metabolomics profiling. Gut microbiota analysis will be performed on stool samples. The assessment of gut microbiota is critical as increasing evidence suggests that some of the health effects of physical activity, sleep, and nutrition may be exerted through or modified via the gut microbiota. Participants' DNA will be obtained to determine genetic variations associated with chronic condition risk factors and telomere length measurement.

Urine Collection

Participants will be invited to collect urine from the time subsequent to going to bed (last void at bedtime not collected), to the first morning void. Urine samples will be received on day 2 (see Urine Sample Collection Instructions). Urine samples will undergo analysis for glucose, albumin, creatinine, melatonin, total protein and metabolomics profiling.

Blood collection

Fasting blood samples will be collected on both days (Day 1 and Day 2); they will be identified by participants' ID and separated as indicated (Table 5). Participants should come in fasting state (at least for 12h) and shouldn't take any alcoholic beverage for at least 48h before each visit. A total of 60 mL of blood will be obtained from participants (Appendix 21). Blood will be drawn by a certified phlebotomist and/or a register nurse.

Stool collection

Participants will be asked to collect stool sample from a bowel movement. After this, they will take samples randomly from 3 different places of the stool. Sample will be given to research personnel at the beginning of second appointment. Research personnel will provide instruction to volunteers at the end of the first visit (see Stool Sample Collection Instructions).

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Urine collection processing and collection instructions

Steps	Processing instructions
1	Receive urine sample and store it directly on 4˚C
2	Aliquot tubes should be labeled with participant ID
3	Number of labels required: 7 – 2.0 ml urine labels (if a urine sample was received)
4	If a urine sample is received proceed as follows: • Determine the volume of the urine • Pour some urine into a sterile container(to keep) • Aliquot urine into 2 -16 x 100 mm tubes and centrifuge
5	Aliquot as follows: 5 cryovials – 2.0 ml / vial (Seven Oaks) 2 cryovials – 2.0 ml / vial (McMillan)
6	Packaging of samples for transport: These samples must not thaw and must arrive frozen at the research lab Pack a transport box with ice packs and the frozen samples .Place the address label on the box. Ask the courier to return the transport box.

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Blood sample processing and collection instructions

Sample	Blood collection tube	Tube volume	Processing instructions	Aliquoting instructions	Analysis	Day
Serum	Red/grey SST tube	1 x 4mL	1. Invert 5 times 2. Room temp for 30 min 3. Spin for 10 min @ 1000 x *g*	1. Aliquot serum into cryovials[1] with brown[2] caps (0.5mL/tube) 2. Store at -80ºC	Insulin Lipid profile Glucose CRP GLP-1	1,2
Plasma	CPT tube (sodium heparin)	1 x 8 mL	1. Invert tube 8- 10 times 2. Spin for 30 min @1500- 1800 RCF 3. Resuspend by inverting 4. After addition of PBS spin for 15 min @ 300 RCF 5. Aspirate off as much supernatant without disturbing the pellet 6. Repeat wash in 10mL PBS 7. Resuspend pellet in 3mL freezing medium -10% DSMO (Sigma), 20% FCS (JRH Bioscience) in RPMI1640 (Gibco)	1. Aliquot entire contents above the gel and transfer to 15 mL Falcon tube 2. Add PBS (w/o Ca++ or Mg++) to make 15 mL 3. Store 1mL aliquots in -70°C using a Cyro-1°C/min freezing container.	T-Regulatory cells*	1
Plasma heparin	Green top (lithium heparin)	1x 4 mL	1. Invert 8 times 2. Spin immediately for 10 min @1300 x *g* 3.	1. Aliquot plasma into cryovials with green[3] caps (0.5mL/tube) 2. Store all fractions at -80ºC	C-reactive protein	1,2
RBC			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot RBC into cryovials with red[5] caps (0.5mL/tube) 2. Store all fractions at -80ºC	Fatty Acid Analysis	1,2
White blood cells Heparin			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot WBC (buffy coat) in 1 (one) Cryo.s™(RNase and DNase free	DNA extraction/ Telomere length	1,2

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Plasma EDTA	Purple top (K2 EDTA)	1X 10 mL	1.Invert 8 timesSpin immediate ly for 10 min@1300 x g 2.After addition of Methanol/ EDTA, spin @ 16,000g for 10 min. @1300 x g	1. Aliquot plasma into cryovials with yellow[5] caps (1.0 mL/tube) 2. Add to 1 plasma aliquot (0.5Ml), 1 volume of sample to 4 volumes of 90% methanol/water/1 mM EDTA 3. Place on dry ice for 5 min 4. Store all fractions at -80ºC	Ascorbic acid	1,2
Plasma EDTA			1.Invert 8 times 2.Spin immediately for 10 min @ 1300 x g	1.Aliquot plasma in cryovials with purple[6] caps (0.5ml/tube) 2.Store all fractions at -80ºC	Leptin Glucagon Oxidized phospholipids and oxylipins	1,2
Plasma EDTA			1.Invert 8 times 2.Spin immediately for 10 min @ 1300 x g	1.Aliquot RBC into cryovials with purple[5] caps (0.5mL/tube) 2.Store all fractions at -80ºC	Non-cholesterol sterols	1,2
White blood cells EDTA			1.Invert 8 times 2.Spin immediately for 10 min @ 1300 x g	Aliquot WBC (buffy coat) in 1 (one) Cryo.s™(RNase and DNase free vials)[4] 2.Store at -80ºC	DNA extraction/ Telomere length	1,2

BMJ Open

The Manitoba Personalized Lifestyle Research (TMPLR) study protocol: a multi-centre bi-directional observational cohort study with administrative health record linkage investigating the interactions between lifestyle and health in Manitoba, Canada

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The Manitoba Personalized Lifestyle Research (TMPLR) study protocol: A multi-centre bi-directional observational cohort study with administrative health record linkage investigating the interactions between lifestyle and health in Manitoba, Canada

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Abstract

Introduction

Lifestyle factors, such as diet, physical activity and sleep, are associated with the development of many chronic diseases. The objective of The Manitoba Personalized Lifestyle Research (TMPLR) study is to understand how these lifestyle factors interact with each other and additional factors, such as an individual's genetics and gut microbiome, to influence health.

Methods

An observational study of adults, with extensive phenotyping by objective health and lifestyle assessments, and retrospective assessment of early life experiences, with retrospective and prospective utilization of secondary data from administrative health records.

Study population

A planned non-random convenience sample of 840 Manitobans aged 30-46 recruited from the general population, stratified by sex (equal males and females), body mass index (BMI; 60% of participants with a BMI >25 kg/m^2), and geography (25% from rural areas,). These stratifications were selected based on Manitoba demographics.

Measurements

Lifestyle factors assessed will include dietary pattern, physical activity, cardiovascular fitness and sleep. Additional factors such as medical history, socio-economic status, alcohol and tobacco consumption, cognition, stress and anxiety, and early life experiences will also be documented. A maternal survey will be performed. Body composition and bone density will be measured by dual energy x-ray absorptiometry. Blood pressure, pulse wave velocity, and augmentation index will be measured on two consecutive days. Chronic disease risk biomarkers will be measured in blood and urine samples. DNA will be extracted for genetic analysis. A fecal sample will be collected for microbiome analysis. Participants may provide their Manitoba Personal Health Information Number (PHIN) to link their study data with administrative health records.

Ethics and dissemination

Ethics approval has been obtained from the University of Manitoba Health Research Ethics Board (protocol # HS18951; 05/01/2016). Data analysis, release of results, and publication of manuscripts are scheduled to start in late 2018. Additional information at www.TMPLR.ca.

Clinicaltrials.gov NCT#xxxxxx

Article Summary

Strengths and limitations of this study

The study is designed to capture extensive phenotyping of participants in the areas of diet, physical activity and sleep, combined with genetic and gut microbiome profiles. The ability to link these study data to healthcare usage data retrospectively and prospectively is a key strength of the research (**Figure 1**). The use of a mobile research unit to access rural populations makes the study unique as geographic setting can strongly influence health-related behaviors.

The study uses non-random convenience sampling for feasibility reasons, which can introduce selection bias and limit generalizability; however, preset stratification based on Manitoba's demographics on sex, BMI, and geography have been implemented so that our final study population will be more representative. Another limitation is that some of the questionnaires used in TMPLR have not previously been validated, or not validated in the specific TMPLR study population. Finally, the study sample size of 840 individuals was not selected to power a specific primary hypothesis; however, it will provide a one-of-a-kind research platform of extensively phenotyped participants in which to investigate the associations between lifestyle and chronic disease.

Introduction

Manitoba is a province located in central Canada with a population of just over 1.2 million people. Most Manitobans (~60%) live in Winnipeg, the largest city, with ~27% of the population living in rural areas [1]. Approximately half of Manitobans are living with at least one of the following chronic conditions: obesity, hypertension, type 2 diabetes (T2D), cardiovascular disease (CVD), or chronic kidney disease (CKD) [2]. Additionally Manitoba has the highest incidence and prevalence of end stage renal disease in Canada, partly because of its high burden of diabetes [2]. The consiquences of these chronic conditions is substantial and the financial burden, both personally and societally, is enormous. In the province of Manitoba, which has a universal healthcare system, over 40 percent of total provincial revenues are spent on healthcare [3]. The burden of conditions including T2D and CKD is not unique to Manitoba [4, 5], therefore the primary and secondary prevention of these chronic conditions is a major international health research priority [6].

It is well established that diet, physical activity, and sleep influence health and mortality [7-10]. Evidence-based guidelines pertaining to nutrition, physical activity and sleep exist to educate the public on healthy lifestyle choices. However, most current lifestyle guidelines follow a one-size-fits-all format, even though they are intended for populations comprising individuals with diverse and complex health circumstances and unique factors influencing their ability to follow the guidelines. This format may be a contributing factor to the poor adherence to lifestyle guidelines. For example, although most people are aware that physical activity is important for health, only 15% of the Canadian population achieve the national recommendations [11]. Similarly, it is estimated that 50% of women and 70% of men in Canada have energy intakes that exceed their energy needs, while 50% to 90% have deficiencies in calcium and vitamin D [12].

There is now an increasing interest in the creation of lifestyle strategies or guidelines for specific sub-populations or groups of individuals with specific characteristics [13-15]. It is hoped that such tailored recommendations will be more effective, and that barriers to healthy lifestyle practices can be ameliorated through personalization. Current one-size-fits-all recommendations and strategies may not be effective due to (1) significant inter-individual variability or (2) shared circumstances, such as geography, sleep/wake pattern, or socio-economic status, of a particular group.

We hypothesize that an individual's lifestyle will be influenced by socio-economic status and geography, and will interact with their genotype and gut microbiota to affect health [16, 17]. Accordingly, The Manitoba Personalized Lifestyle Research (TMPLR) study will involve the coordinated collection of data related to socio-economic status, geography, nutrition, physical activity, sleep, early life experiences, and health systems usage, in conjunction with the analysis of genetics, gut microbiota, and risk factors for chronic conditions such as obesity, hypertension, T2D, CVD, and CKD. After establishing the baseline characteristics of this study cohort, administrative health records will be used retrospectively to examine the developmental origins of health and disease [18], and prospectively to track and investigate the development of chronic disease in the future, starting at 5 years after the initial study is complete. Consent will be obtained to contact study participants for further clinical assessments, contingent on future funding.

Data from this study will provide an ideal opportunity for the exploration and potential discovery of new interactive mechanisms through which lifestyle factors affect health. We will be looking

to collaborate with other existing studies [19-21]with overlapping measures to replicate such findings, or increase sample size.. Findings from this research may be useful in guiding both clinical and health policy decisions, and will also facilitate the design and testing of personalized health promotion strategies. For example, if we are able to identify interactions between lifestyle factors and disease risk, such as a genetic variant that associates with short sleep to negatively impact health, a follow-up study could be designed looking to improve sleep hygiene specifically in the group with the risk variant.

Methods

Design

This is an exploratory observational cohort study with retrospective and prospective utilization of secondary data from administrative health records (**Figure 1**). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed where applicable in the development of this protocol manuscript [22].

Setting

Urban (Winnipeg) and rural (Morden, Winkler, Carman, Steinbach) areas with road access in southern Manitoba, Canada.

Objectives of the study

The objective of this study is to explore the complex interactions that exist between lifestyle, genetics, and gut microbiota, and how these relate to risk factors for chronic conditions, especially obesity, hypertension T2D, CVD, and CKD in Manitoba.

Inclusion and exclusion criteria

A sample of 800 Manitobans aged 30-46, stratified by sex, BMI, and geography **(Table 1)** are being recruited. Participants must have lived in Manitoba for a minimum of 5 years. Women who are pregnant or lactating are not eligible to participate. Additionally, because it is expected that very few of the 800 Manitobans who join TMPLR study from the general public will have reduced kidney function (eGFR <30 ml/min), 40 participants from Manitoba (20 female, 20 male, with no set stratification based on BMI or geography) who have severely reduced kidney function are being recruited from the renal health clinic at Seven Oaks General Hospital (SOGH), Winnipeg, MB. Therefore, the study has a recruitment goal of 840 participants.

Recruitment

Participants are recruited through the use of printed flyers, online advertisements purchased via Google, Facebook, and Twitter ad platforms and social media accounts, appearances in local TV, radio, and print media, and direct contact with community groups, such as churches, sports leagues, and community clubs. All patients who receive care in the SOGH renal health clinic, who are aged 30-46, have been living in Manitoba for a minimum of the last 5 years, and are able to provide informed consent are approached to enroll in the study as well.

Sample size

The sample size of TMPLR study was selected based on considerations of feasibility of recruitment, costs, and logistics. However, given established values from other sources [23] and our anticipated sample size of 840 participants, we estimate that we will have an 80% power (5% significance, 2-sided) to detect a minimum body fat difference of 2.5% for rare exposures (i.e.,

experienced by 10% of participants, such as smoking) and 1.7% for more common exposures (experienced by 25% of participants, such meeting the Canadian recommended 150 minutes of moderate-to-vigorous physical activity). Additional estimated minimum detectable differences are presented in **Table 2**. These lower limits should allow for the detection of clinically meaningful changes in these outcomes.

Data Collection and Assessments

On two consecutive days, participants come to either the urban TMPLR study site at the Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba or TMPLR's mobile research unit which travels to other areas of Winnipeg and southern Manitoba. TMPLR's mobile research unit is a custom built 12-meter mobile lab which is equipped with phlebotomy area, a dual-energy X-ray absorptiometer (DXA) and a bicycle ergometer with a metabolic cart. During this visit, participants complete questionnaires, undergo various health assessments, provide urine and fecal samples, and have fasting blood samples taken **(Figure 2, Table 3)**. The same protocols at were followed at both sites.

Questionnaires

Questionnaires capture socio-demographic characteristics, personal and family medical history, smoking (including electronic cigarette use), current diet (three Automated Self-Administered 24-h (ASA24) Dietary Assessment Tool recalls , Mindful Eating Questionnaire [24], Diet History Questionnaire [25] and The Three-Factor Eating Questionnaire [26]), alcohol consumption, physical activities, frailty using the Modified Fried Criteria [27], stress, sleep (Pittsburgh Sleep Quality Index [28]), cognition (Montreal Cognitive Assessment Questionnaire [29]), and childhood retrospective circumstances (adapted from the US Panel Study on Income Dynamics [30]).

Anthropometric assessment

Weight is measured after participants change into lightweight scrub tops and bottoms, with shoes removed, to the nearest 0.1 kg using a digital calibrated floor scale (7562EF, Taylor Precision Products, Oak Brook, IL, USA). Height is measured, without shoes, to the nearest 0.1 cm using a stadiometer (Model 206, SECA North America, Chino, CA, USA). BMI is calculated in kg/m^2. Waist circumference is measured in triplicate, to the nearest 0.1 cm at the umbilicus, between the last rib and the iliac crest using a fibreglass tape measure. Hip circumference is measured in triplicate at the widest portion of the buttocks and hips using a fibreglass tape measure. Body composition including fat mass, lean mass, percent body fat, visceral adipose tissue (VAT), and bone mineral density (BMD) are assessed using dual-energy X-ray absorptiometry (DXA, Lunar Prodigy Advance, GE Healthcare, Mississauga, ON, CAN) [31]. Scans are taken of the whole body, femoral neck, L1-L4 of the spine, and the non-dominant forearm.

Clinical health assessment

Participants' systolic and diastolic blood pressures are measured in triplicate, on the non-dominant arm in a sitting position using a validated oscillometric blood pressure monitor (BP760CAN, Omron, Burlington, ON, CAN). Participants are required to rest for 5-10 minutes before taking the measurement. Pulse wave velocity and augmentation index are measured on the non-dominant arm in a sitting position using a Mobil-O-Graph PWA Monitor and the HMS

Client Server Software (I.E.M Gmbh, Stolberg, Germany) according to the manufacturer's protocol on two consecutive days [32].

Collection of bio-specimens

Blood, urine, and fecal samples are obtained from study participants **(Supplementary protocols)**. Fasting blood samples are collected on two consecutive days via venipuncture by trained phlebotomists. Two blood samples on consecutive days are required to undertake the isotopic assessment of fractional cholesterol and triglyceride synthesis rates. Participants are asked to collect two urine samples at home; one sample is obtained prior to going to bed, and a second of the first morning void upon waking up. Participants also collect a fecal sample; they are provided a collection kit and instructed to collect a single sample from 3 separate places on the stool using a spoon attached to the cap of the collection tube. Participants are instructed to store the collected samples fecal in their household –20°C freezer with a provided ice pack, and urine samples in the fridge, until transport back to the study center, using provided ice pack for temperature control, where they are aliquoted and then stored at –80°C for future analysis [33].

Clinical chemistry in blood and urine

Clinical chemistry, including lipid profile, glucose, insulin, and renal and liver profiles will be measured via automated clinical chemistry analyzers (Cobas C111, C311 and e411, Roche Diagnostics Laval, QC). Additional blood and urine biomarkers such as leptin, glucagon, and melatonin will be measured via ligand binding assay (LBA) or enzyme-linked immunosorbent assay (ELISA). Red blood cell and plasma fatty acids will be measured by gas chromatography with flame ionization detections (GC-FID)[34]. Non-cholesterol sterols will be measured in plasma using GC-FID and mass spectrometry (MS) [35]. Vitamin C concentrations in the blood will be measure by high pressure liquid chromatography (HPLC) [36].

Microbiome analyses in fecal samples

Fecal samples will be subjected to genomic DNA extraction (Zymo Research, CA, USA) following the manufacturer's protocol. Experimental negative controls will be included in extraction protocols to confirm the reliability and consistency of the extracted nucleic acid. The V4 hypervariable region of 16S rRNA gene will be amplified, the sequencing library will be generated as described previously [37] and sequenced at the Gut Microbiome Laboratory, University of Manitoba. Samples will be multiplexed at the rate of 200 per run aiming for an average sequencing depth of 50,000 sequences per sample. The sequencing data will be deposited into the Sequence Read Archive (SRA) of NCBI (http://www.ncbi.nlm.nih.gov/sra) and accession numbers will be provided for future access.

Deuterium oxide administration

After the blood sample collection on day one, participants are given 0.7 grams of deuterium oxide per kilogram of estimated body water to drink. Body water is estimated as body weight (kg) x 0.60. This deuterium administration is used to enrich the body's water pool for the assessment of fractional cholesterol and triglyceride synthesis rates [38-40].

Physical activity and capacity testing

Physical activity level in TMPLR study participants is assessed using accelerometers (Actigraph GTX3bt, Penscola. FL, USA) worn for 1 week [41, 42]. Muscle strength is measured using a hand grip dynamometer. Cardiorespiratory fitness is assessed using a submaximal bike protocol which includes heart rate monitoring, and a metabolic cart (VMAX Encore, Carefusion, Unionville, Ont., Canada) to measure oxygen consumption and CO_2 output. Functional walking ability is assessed using a 5-meter gait speed test. Additionally, depressive symptoms, obesity history, frailty, low physical activity, and cognitive impairment are assessed by validated questionnaires [43-45].

Sleep assessment

Sleep in TMPLR study participants is measured objectively using accelerometers (Actigraph GTX3bt, Penscola. FL, USA [46]) worn for a week and subjectively by questionnaire (Pittsburgh Sleep Quality Index [28]). While there is a strong relationship between objective and subjective sleep reports, TMPLR study is collecting both because discrepancies may provide important clinical information reflecting early dysfunction [47, 48].

Dietary assessment

Study participants complete the Canadian version of the Diet History Questionnaire (DHQ) [25], which estimates the intake of common food items and includes portion size and dietary supplement questions. This questionnaire is on a TELEform for scanning data entry and creation of the data files. Participants also complete the Mindful Eating Questionnaire [24] to assess awareness of the physical and emotional sensations associated with eating, and The Three-Factor Eating Questionnaire [26] to assess dietary restraint, disinhibition and hunger in relation to eating. Participants also complete three dietary recall surveys using the Automated Self-Administered 24-hour Canada (ASA24®, NCI, Rockville, Maryland U.S. http://asa24.ca/)[49] dietary assessment tool, a web-based tool that enables multiple, automatically coded, self-administered 24-hour recalls. Participants enrolled from March 2016 to February 2017 used the ASA24-Canada-2014 edition; those enrolled after February 2017 used the ASA24-Canada-2016 edition. Both ASA24-Canada-2014 and ASA24-Canada-2016 use the same nutrient databases.

Early life experiences

Early-life exposures spanning the critical time windows of fetal development, birth, infancy and early childhood are documented in three ways: 1) through linkage with administrative health records (see Linkage to administrative health data), 2) by self-report, and 3) by maternal report. Administrative health data will provide method of birth, gestational age, birth weight, diagnosis codes for post-delivery hospitalization, and post-delivery drug prescriptions. Mothers of TMPLR study participants are asked to complete a TMPLR Mother's Questionnaire, adapted from the Nurses' Health Study [50], capturing key pregnancy, birth, and postpartum events such as method of birth; gestational age and birth weight; socioeconomic status at birth; maternal pre-pregnancy BMI and gestational weight gain; maternal smoking and diabetes during pregnancy; maternal prenatal care; breastfeeding initiation, exclusivity and duration; stressful life events during pregnancy and postpartum; and severe illness requiring hospitalization during infancy or early childhood. Early childhood socioeconomic status [51, 52] and stressful life events [53, 54] are also self-reported by TMPLR participants using the Childhood Retrospective Circumstances Questionnaire, adapted from the US Panel Study of Income Dynamics [30].

Data quality assurance and control

Methods of data collection (questionnaires, anthropometric assessment, and clinical health assessment) were standardized across the urban and mobile TMPLR study sites. Training of TMPLR study staff involved in data collection and data entry is regularly refreshed and all staff handling participant data are trained in compliance with the Manitoba Personal Health Information Act (PHIA). All data will be entered in the secure digital platform. A TMPLR study data model has been created to help in visualizing the different types of data the digital platform will contain. (**Supplementary figure 1)**

Linkage to administrative health data

At enrollment, TMPLR participants are asked to provide their PHIN and grant permission to link their study data with administrative health records (including hospital discharge abstracts, physician billing claims, and prescription records). These data are accessed through the Manitoba Centre for Health Policy (MCHP) Population Research Data Repository [55] and linkage is achieved using the PHIN, following the standard procedures established by the MCHP and the Manitoba Health Information Privacy Committee. The data linkage is used to capture retrospective information on early life as well as prospective information on numerous health outcomes, including diagnosis of hypertension, T2D, CVD, and CKD.

Statistical analyses

Statistical analyses will be undertaken in consultation with biostatisticians from the George and Fay Yee Centre for Healthcare Innovation (CHI) at the University of Manitoba. Lifestyle factors will primarily be used as explanatory variables, with chronic disease biomarkers or disease presence/absence as outcomes, in multivariable regression models. Moderating or mediating effects of genetics, gut microbiome, clinical characteristics, socio-economic status, and environmental factors will be explored. The potential confounding effects of health status and healthcare use on variable relationships will be examined using techniques such as propensity score or instrumental variable models [56-58].

Techniques appropriate for high-dimensional data will be adopted where needed. For example, clustering of lifestyle risk factors will be examined using latent variable modeling techniques (i.e., latent class analysis). Dimension reduction techniques for omics data, such as microbiome and genetic markers, will be applied [59].

The bioinformatics and statistical analyses of microbiome data will be performed as described previously [37] and will be updated based on recommendations and technology advancements between now and the point of processing of samples. Overall microbiota community structures, alpha diversity metrics, and relative abundances of operational taxonomic units (OTUs), will be tested for associations with lifestyle and health measures, with appropriate adjustment for multiple comparisons.

Non-response bias may affect the validity of analyses for survey data, necessitating the use of multiple imputation methods if the pattern of missing data is deemed to be ignorable [60]. For non-ignorable missing data, selection and pattern mixture models will be examined in sensitivity analyses [61]. Due to the use of non-random sampling there is a risk of selection bias; survey weights and weighting of responses may be used to address this bias. Standardization or adjustment techniques may be used to address bio-specimen measurement error bias [62].

Specialized methodological investigations will be conducted for: 1) psychometric analyses of scales, including testing for differential item functioning and measurement invariance [63-65], 2) development of chronic disease risk prediction models [66, 67], 3) techniques to evaluate the quality of linked databases, including their accuracy, reliability, and completeness [68], 4) robust statistical methods for the analysis of outcome measures with non-normal (e.g. skewed) distributions [69, 70].

'Patient and Public Involvement Three focus group, one for healthcare providers and two for general public, and a public forum were held in the early design stages of this study to obtain input from Manitobans, on the study design and recruitment strategies. A study advisory board was also formed, and meets on a bi-annual basis. This advisory board includes healthcare providers, health researchers, and members of the public. The board provides input regarding study recruitment, progress and conduct, and will also provide input and suggestions regarding the dissemination of study results.

Provision of clinical results to participants

Individual results of the anthropomorphic measurements, blood pressure, pulse wave velocity, augmentation index, body composition, bone density, full lipid profile, fasting blood glucose, and renal and liver profile are to be provided to participants. Participants are referred to their primary care providers for further management if their results are beyond clinical reference ranges. Participants will not be provided their genetic and microbiome information.

Ethics and dissemination

Explicit informed consent is obtained from each individual prior to participation in the study. Eligible participants are verbally informed by trained research personnel regarding the nature and purpose of the study, given time to decide whether or not to participate, and have any questions or concerns answered prior to consent and at any point throughout the study. All participants are informed that they may withdraw from the study at any time without penalty and are remunerated for the portion of the study that they have completed up to that point. The full remuneration for study participation is $100 Canadian dollars, provided as cash or as a gift card.

Ethics approval has been obtained from the University of Manitoba Health Research Ethics Board prior to participant recruitment (protocol# HS18951). The study protocol has also been reviewed and approved by the Manitoba Health Information Privacy Committee in regards to the collection and use of PHIN, The St. Boniface Hospital Research Review Committee in regards to the processing of samples at the hospital, and the Winnipeg Regional Health Authority (WRHA) Research Access and Approval Committee (RAAC), the Southern Health Research Ethics Board, and the Interlake-Eastern Regional Health Authority Regional Ethics Committee, in regards to the study taking place in those health regions.

Data analysis, release of results, and publication of initial manuscripts are scheduled for 2019. Findings will be shared in peer-reviewed journals, and at regional, national, and international scientific conferences. Data and findings will also be presented to healthcare policymakers within Manitoba, to develop preventive strategies that reduce chronic conditions with the intention of reducing healthcare costs. Funding applications for future clinical follow in this study population have been submitted starting in 2017**.**

Discussion

TMPLR study has been uniquely designed to provide cross-sectional, retrospective and prospective observations that will improve our understanding of how lifestyle factors interact with each other and additional factors such as genetics and the gut microbiome to influence health and the risk of obesity, T2D, CVD, and CKD**.** The coordinated collection of lifestyle-gene-environment-microbiota-health data, including objective measurements such as DXA, activity monitoring, stable isotopic tracer methodologies, and direct measurement of physiological biomarkers; combined with the ability to retrospectively assess and prospectively follow health outcomes in participants using administrative health records, represents an unprecedented opportunity to collect data which can be used to improve chronic disease prevention and management.

Due to the voluntary non-random recruitment of participants, there may be an under-representation of those with lower health awareness, financial means, access, or time to participate. Attempts to counteract this are implicit in the stratified recruitment design. Comparisons between TMPLR study participants and general Manitoban population demographics may allow assessment of potential selection biases. A healthy volunteer effect may impact the ability to detect weak associations between lifestyle and disease risk, but this may attenuate with longer follow-up using administrative health data.

Given a projected sample size of 840 participants may be low for some of research questions that will be investigated, therefore harmonization and linking of data across multiple cohorts may be required. We will be looking to other studies which have undertaken overlapping measurements in order to increase sample sizes. The Canadian Longitudinal Study on Aging [19], the Toronto Nutrigenomics and Health [20], and The LifeLines DEEP [21] studies among others will be approached regarding the potential of data harmonization and cross-replication. TMPLR study will also be available to other researchers who are interested in collaboration or using the data for cross-replication.

In summary, TMPLR study will provide a unique platform of extensively phenotyped individuals that will be used to explore the interactions between lifestyle factors that associate with the development of, or protection from, obesity, hypertension, T2D, CVD, and CKD. The findings from this research platform will subsequently be used to develop and test preventive and restorative lifestyle and health strategies with the aim of improving the health and reducing healthcare costs at the individual and population levels.

Study status

Data collection started in March 2016. As of the August 15th 2018, data collection has been completed for over 800 participants.

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Author contributions

DSM and RCM developed the original concept of the study for the original grant application with input from co-investigators. DSM prepared the drafts of the study protocol manuscript and compiled feedback and changes from other authors. MG assisted in the preparation of the study protocol manuscript. PF developed the branding for TMPLR study, and the manuscript figures and tables. NM prepared the data model and was involved in the public engagement. SB (project lead, indigenous health), HB (project lead, nutrition), JC, TAD (project lead, physical activity), PKE (project lead, genetics), EK (project lead, gut microbiome), LML (project lead, biostatistics), DEM (project lead, sleep), SBM, AR, NT, MBA (project lead, developmental origins of chronic disease), and PJJ (Director) are study co-investigators, and were all involved in writing the original grant application. All authors have carefully read, contributed to, and approved the final version of the study protocol manuscript.

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Competing interests statement

DSM, RCM, MG, SB, HB, JC, TAD, PKE, PF, NH, EK, LML, DEM, SBM, AR, MBA, and PJJ have no competing interests to declare.

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Figure 1. The Manitoba Personalized Lifestyle Research Study overview

Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule

Table 1. The Manitoba Personalize Lifestyle Research Study recruitment targets by strata

Age	30-46 years N=800	
Sex	400 Males	400 Females

50% Male 50% Female								
Geography 72% Urban 28% Rural	288 Urban males		112 Rural males		288 Urban females		112 Rural females	
BMI 40% Normal (BMI <25) 60% Overweight (BMI ≥ 25)	116 Urban males BMI <25	172 Urban males BMI ≥ 25	45 Rural males BMI <25	67 Rural males BMI ≥ 25	116 Urban females BMI <25	172 Urban females BMI ≥ 25	45 Rural females BMI <25	67 Rural females BMI ≥ 25
+40 participants with severely reduced kidney function (eGFR <30 ml/min), 20 female, 20 male, with no set stratification based on BMI or geography								

Table 2. The Manitoba Personalized Lifestyle Research (TMPLR) study estimated minimum detectable differences

Variable	Mean or median used	Standard deviation used	Minimum difference at 10% exposure (percentage of mean)	Minimum difference at 25% exposure (percentage of mean)	References
Body fat (%)	41.3% Females 27.8% Males	7.7% 6.6%	2.5% (6.0)	1.7% (4.0)	[23]
Lumbar bone mineral density (BMD; g/cm^2)	1.042 Females 1.058 Males	0.121 0.127	0.041 (3.8)	0.028 (2.6)	[71]
Glomerular filtration rate (GRF; ml/min per 1.73 m^2)	107.6	16.8	5.4 (5.0)	3.8 (3.5)	[72]

Systolic blood pressure (mmHg)	116	12	6.5 (5.6)	4.5 (3.9)	[73]
Fasting Glucose (mmol/L)	4.94	0.61	0.20 (4.0)	0.14 (2.8)	[73]
Fasting insulin (µIU/mL)	7.83	7.50	2.40 (30)	1.67 (21%)	[74]
LDL cholesterol (mmol/L)	2.79	0.67	0.22 (7.8)	0.15 (5.4)	[73]
Waist circumference (cm)	80	10	3.2 (4)	2.2 (2.75)	[73]

Table 3. The Manitoba Personalized Lifestyle Research (TMPLR) study data, assessment tools and biological samples

Characteristic	***Data***	***Method, Instrument or Source***
Sociodemographic	Date of birth, Sex, Ethnicity, Marital status	TMPLR study questionnaire
Medical	Personal medical history, Family medical history, Medication(s), Pregnancy history	TMPLR study questionnaire, Administrative health records
	Cognition	Montreal Cognitive Assessment [29]
Lifestyle	Tobacco/smoking/vaping use, Alcohol use, Unintentional weight loss, Exhaustion, Depression	TMPLR study questionnaire
Physical activity	Frailty	Modified Fried Criteria [27]
	Physical activity	Paffenbarger physical activity index, Actigraphy [41, 42]
	Predicted VO2 max	Modified YMCA bike test with metabolic cart
Nutrition	Dietary patterns and habits	Mindful eating questionnaire[24], three-factor eating questionnaire [26], automated 24-hour dietary recall [49], Canadian dietary history questionnaire [25]
Early life	Childhood health, socio-demographic and socioeconomic status; Parental employment history	Childhood retrospective questionnaire, adapted from the US Panel Study on Income Dynamics[30]

	Maternal: pregnancy events, obstetrical history, infant feeding	TMPLR Mother's retrospective childhood questionnaire, adapted from the Nurses Health Study [50]
Socioeconomic	Employment, Home ownership, Education attainment, Income	TMPLR study questionnaire
Sleep and Stress	Duration of sleep	Actigraphy [46]
	Sleep Quality	Pittsburgh sleep quality index [28]
	Perception of stress, Daily life stressors	Community-based stress and coping survey
Anthropometric	Height	Wall-mounted stadiometer
	Weight	Digital scale
	Waist circumference, Hip circumference	Tape measure
	Body fat, Lean mass, bone mineral density	Dual energy X-ray absorptiometry [31]
Blood pressure	Systolic & diastolic	Automated sphygmomanometer
	Pulse wave velocity, Augmentation index	Mobil-O-Graph oscillometer [32]
Biomarkers	Blood clinical chemistry and biomarker assays	Fasting blood samples
	Urinary clinical chemistry and biomarker assays	Urine samples
	Microbiome 16S RNA sequencing	Fecal sample [37]

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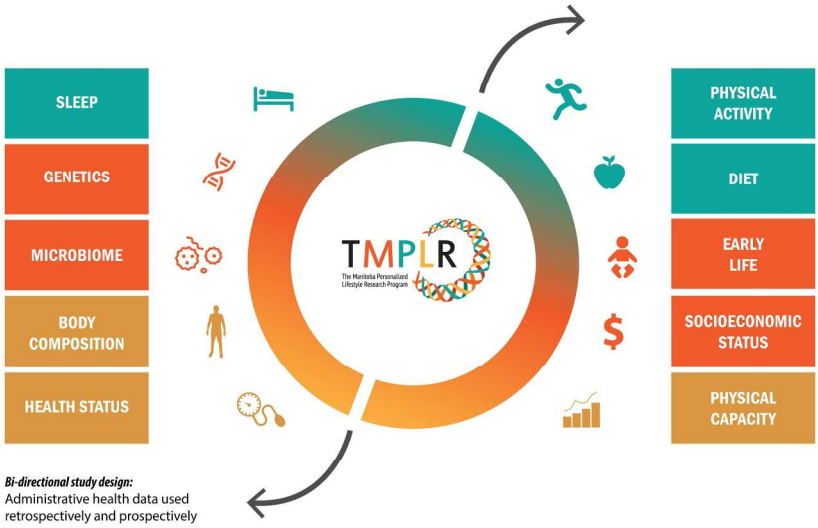

Figure 1. The Manitoba Personalized Lifestyle Research Study overview

230x130mm (300 x 300 DPI)

Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule

279x361mm (300 x 300 DPI)

TMPLR Data Model

Linking key: TMPLR ID #

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Urine Sample Collection Instructions

Please follow these instructions for urine collection. Research personnel will provide you with 2 urine collection cups labeled with time (night and day) and your TMPLR Study ID Number.

1. **Check your study ID on the collection tubes.** If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the samples.

2. Collect urine before going to bed tonight in the cup labeled "night". **Please write down the date and time of the sample was collected**. Store the sample in the fridge in the Ziploc bag provided.

3. Collect urine from the first time you pee after getting up in the morning, in the cup labeled "day". **Please write down the date and time of the sample was collected**. Store your samples in the fridge in the Ziploc bag provided.

4. Please bring the urine samples with you on your day 2 visit. TMPLR staff will collect the samples from you when you arrive.

If you have any questions, please contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.

Thank you for your cooperation!

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The Manitoba Personalized Lifestyle Research (TMPLR) Study

Stool Sample Collection Instructions

1. Freeze the ice packs provided by the study once you get home.

2. **Check your study ID** on the collection tubes (the two plastic tubes with blue cap). If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the sample.

3. Empty your bladder. Flush toilet. Place the collection unit under the rear part of the toilet seat with the round side pointing towards the back.

4. Have a bowel movement. **Collect 2 samples, one in each plastic tube, from 3 different places of the stool** using the spoon attached to the cap of the collection tubes. **Fill each sample tube about one third of the tube with stool sample**.

5. Close the tube tightly. Place each tube in a Ziploc bag provided. **Write down the date and time of the bowel movement** on the bag. Discard the used collection unit.

6. Wrap the collection tubes with the frozen ice packs, and keep them in the paper bag provided. Keep the collected sample in the freezer.

7. Return the stool samples wrapped with the frozen ice packs on day 2 of the measurements, or as soon as you can. TMPLR staff will collect the sample from you in the paper bag when you arrive.

If you have any questions, feel free to contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.

Collection Unit

Collection Tube with Spoon

Biospecimens collection

Blood samples will undergo analysis for numerous established and emerging health biomarkers, these include: total cholesterol, LDL-C, HDL-C, triglycerides, glucose, AST, ALT, insulin, glucagon-like peptide-1(GLP-1), leptin, c-reactive protein (CRP), fatty acids, HbA1c, T-regs, serum creatinine, blood urea nitrogen (BUN), non-cholesterol sterols, adipokines, cytokines,vitamin C, fat soluble vitamins, and lipidomic and metabolomics profiling. Gut microbiota analysis will be performed on stool samples. The assessment of gut microbiota is critical as increasing evidence suggests that some of the health effects of physical activity, sleep, and nutrition may be exerted through or modified via the gut microbiota. Participants' DNA will be obtained to determine genetic variations associated with chronic condition risk factors and telomere length measurement.

Urine Collection

Participants will be invited to collect urine from the time subsequent to going to bed (last void at bedtime not collected), to the first morning void. Urine samples will be received on day 2 (see Urine Sample Collection Instructions). Urine samples will undergo analysis for glucose, albumin, creatinine, melatonin, total protein and metabolomics profiling.

Blood collection

Fasting blood samples will be collected on both days (Day 1 and Day 2); they will be identified by participants' ID and separated as indicated (Table 5). Participants should come in fasting state (at least for 12h) and shouldn't take any alcoholic beverage for at least 48h before each visit. A total of 60 mL of blood will be obtained from participants (Appendix 21). Blood will be drawn by a certified phlebotomist and/or a register nurse.

Stool collection

Participants will be asked to collect stool sample from a bowel movement. After this, they will take samples randomly from 3 different places of the stool. Sample will be given to research personnel at the beginning of second appointment. Research personnel will provide instruction to volunteers at the end of the first visit (see Stool Sample Collection Instructions).

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Urine collection processing and collection instructions

Steps	Processing instructions
1	Receive urine sample and store it directly on 4˚C
2	Aliquot tubes should be labeled with participant ID
3	Number of labels required: 7 – 2.0 ml urine labels (if a urine sample was received)
4	If a urine sample is received proceed as follows: • Determine the volume of the urine • Pour some urine into a sterile container(to keep) • Aliquot urine into 2 -16 x 100 mm tubes and centrifuge
5	Aliquot as follows: 5 cryovials – 2.0 ml / vial (Seven Oaks) 2 cryovials – 2.0 ml / vial (McMillan)
6	Packaging of samples for transport: These samples must not thaw and must arrive frozen at the research lab Pack a transport box with ice packs and the frozen samples .Place the address label on the box. Ask the courier to return the transport box.

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Blood sample processing and collection instructions

Sample	Blood collection tube	Tube volume	Processing instructions	Aliquoting instructions	Analysis	Day
Serum	Red/grey SST tube	1 x 4mL	1. Invert 5 times 2. Room temp for 30 min 3. Spin for 10 min @ 1000 x *g*	1. Aliquot serum into cryovials[1] with brown[2] caps (0.5mL/tube) 2. Store at -80ºC	Insulin Lipid profile Glucose CRP GLP-1	1,2
Plasma	CPT tube (sodium heparin)	1 x 8 mL	1. Invert tube 8- 10 times 2. Spin for 30 min @1500- 1800 RCF 3. Resuspend by inverting 4. After addition of PBS spin for 15 min @ 300 RCF 5. Aspirate off as much supernatant without disturbing the pellet 6. Repeat wash in 10mL PBS 7. Resuspend pellet in 3mL freezing medium -10% DSMO (Sigma), 20% FCS (JRH Bioscience) in RPMI1640 (Gibco)	1. Aliquot entire contents above the gel and transfer to 15 mL Falcon tube 2. Add PBS (w/o Ca++ or Mg++) to make 15 mL 3. Store 1mL aliquots in -70°C using a Cyro-1°C/min freezing container.	T-Regulatory cells*	1
Plasma heparin	Green top (lithium heparin)	1x 4 mL	1. Invert 8 times 2. Spin immediately for 10 min @1300 x *g* 3.	1. Aliquot plasma into cryovials with green[3] caps (0.5mL/tube) 2. Store all fractions at -80ºC	C-reactive protein	1,2
RBC			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot RBC into cryovials with red[5] caps (0.5mL/tube) 2. Store all fractions at -80ºC	Fatty Acid Analysis	1,2
White blood cells Heparin			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot WBC (buffy coat) in 1 (one) Cryo.s™(RNase and DNase free	DNA extraction/ Telomere length	1,2

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Plasma EDTA	Purple top (K2 EDTA)	1X 10 mL	1.Invert 8 timesSpin immediate ly for 10 min@1300 x g 2.After addition of Methanol/ EDTA, spin @ 16,000g for 10 min. @1300 x g	1. Aliquot plasma into cryovials with yellow[5] caps (1.0 mL/tube) 2. Add to 1 plasma aliquot (0.5Ml), 1 volume of sample to 4 volumes of 90% methanol/water/1 mM EDTA 3. Place on dry ice for 5 min 4. Store all fractions at -80ºC	Ascorbic acid	1,2
Plasma EDTA			1.Invert 8 times 2.Spin immediately for 10 min @ 1300 x g	1.Aliquot plasma in cryovials with purple[6] caps (0.5ml/tube) 2.Store all fractions at -80ºC	Leptin Glucagon Oxidized phospholipids and oxylipins	1,2
Plasma EDTA			1.Invert 8 times 2.Spin immediately for 10 min @ 1300 x g	1.Aliquot RBC into cryovials with purple[5] caps (0.5mL/tube) 2.Store all fractions at -80ºC	Non-cholesterol sterols	1,2
White blood cells EDTA			1.Invert 8 times 2.Spin immediately for 10 min @ 1300 x g	Aliquot WBC (buffy coat) in 1 (one) Cryo.s™(RNase and DNase free vials)[4] 2.Store at -80ºC	DNA extraction/ Telomere length	1,2

BMJ Open

The Manitoba Personalized Lifestyle Research (TMPLR) study protocol: a multi-centre bi-directional observational cohort study with administrative health record linkage investigating the interactions between lifestyle and health in Manitoba, Canada

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The Manitoba Personalized Lifestyle Research (TMPLR) study protocol: A multi-centre bi-directional observational cohort study with administrative health record linkage investigating the interactions between lifestyle and health in Manitoba, Canada

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Abstract

Introduction

Lifestyle factors, such as diet, physical activity and sleep, are associated with the development of many chronic diseases. The objective of The Manitoba Personalized Lifestyle Research (TMPLR) study is to understand how these lifestyle factors interact with each other and other factors, such as an individual's genetics and gut microbiome, to influence health.

Methods

An observational study of adults, with extensive phenotyping by objective health and lifestyle assessments, and retrospective assessment of early life experiences, with retrospective and prospective utilization of secondary data from administrative health records.

Study population

A planned non-random convenience sample of 840 Manitobans aged 30-46 recruited from the general population, stratified by sex (equal males and females), body mass index (BMI; 60% of participants with a BMI >25 kg/m^2), and geography (25% from rural areas,). These stratifications were selected based on Manitoba demographics.

Measurements

Lifestyle factors assessed will include dietary pattern, physical activity, cardiovascular fitness and sleep. Factors such as medical history, socio-economic status, alcohol and tobacco consumption, cognition, stress and anxiety, and early life experiences will also be documented. A maternal survey will be performed. Body composition and bone density will be measured by dual energy x-ray absorptiometry. Blood pressure, pulse wave velocity, and augmentation index will be measured on two consecutive days. Chronic disease risk biomarkers will be measured in blood and urine samples. DNA will be extracted for genetic analysis. A fecal sample will be collected for microbiome analysis. Participants may provide their Manitoba Personal Health Information Number (PHIN) to link their study data with administrative health records.

Ethics and dissemination

Ethics approval has been obtained from the University of Manitoba Health Research Ethics Board (protocol # HS18951; 05/01/2016). Data analysis, release of results, and publication of manuscripts are scheduled to start in early 2019. Additional information at www.TMPLR.ca.

Clinicaltrials.gov NCT03674957

Article Summary

Strengths and limitations of this study

- The study is designed to capture extensive phenotyping of participants in the areas of diet, physical activity and sleep, genetic and gut microbiome profiles, and healthcare usage data linkage.
- The use of a mobile research unit to access rural populations makes the study unique as geographic setting can strongly influence health-related behaviors. The study uses non-random convenience sampling for feasibility reasons, which can introduce selection bias and limit generalizability.
- Some of the questionnaires used in TMPLR have not previously been validated, or not validated in the specific TMPLR study population.
- The study sample size of 840 individuals was not selected to power a specific primary hypothesis and therefore should be considered exploratory in nature.

Introduction

Manitoba is a province located in central Canada with a population of just over 1.2 million people. Most Manitobans (~60%) live in Winnipeg, the largest city, with ~27% of the population living in rural areas [1]. Approximately half of Manitobans are living with at least one of the following chronic conditions: obesity, hypertension, type 2 diabetes (T2D), cardiovascular disease (CVD), or chronic kidney disease (CKD) [2]. Additionally Manitoba has the highest incidence and prevalence of end stage renal disease in Canada, partly because of its high burden of diabetes [2]. The consiquences of these chronic conditions is substantial and the financial burden, both personally and societally, is enormous. In the province of Manitoba, which has a universal healthcare system, over 40 percent of total provincial revenues are spent on healthcare [3]. The burden of conditions including T2D and CKD is not unique to Manitoba [4, 5], therefore the primary and secondary prevention of these chronic conditions is a major international health research priority [6].

It is well established that diet, physical activity, and sleep influence health and mortality [7-10]. Evidence-based guidelines pertaining to nutrition, physical activity and sleep exist to educate the public on healthy lifestyle choices. However, most current lifestyle guidelines follow a one-size-fits-all format, even though they are intended for populations comprising individuals with diverse and complex health circumstances and unique factors influencing their ability to follow the guidelines. This format may be a contributing factor to the poor adherence to lifestyle guidelines. For example, although most people are aware that physical activity is important for health, only 15% of the Canadian population achieve the national recommendations [11]. Similarly, it is estimated that 50% of women and 70% of men in Canada have energy intakes that exceed their energy needs, while 50% to 90% have deficiencies in calcium and vitamin D [12].

There is now an increasing interest in the creation of lifestyle strategies or guidelines for specific sub-populations or groups of individuals with specific characteristics [13-15]. It is hoped that such tailored recommendations will be more effective, and that barriers to healthy lifestyle practices can be ameliorated through personalization. Current one-size-fits-all recommendations and strategies may not be effective due to (1) significant inter-individual variability or (2) shared circumstances, such as geography, sleep/wake pattern, or socio-economic status, of a particular group.

We hypothesize that an individual's lifestyle will be influenced by socio-economic status and geography, and will interact with their genotype and gut microbiota to affect health [16, 17]. Accordingly, The Manitoba Personalized Lifestyle Research (TMPLR) study will involve the coordinated collection of data related to socio-economic status, geography, nutrition, physical activity, sleep, early life experiences, and health systems usage, in conjunction with the analysis of genetics, gut microbiota, and risk factors for chronic conditions such as obesity, hypertension, T2D, CVD, and CKD. After establishing the baseline characteristics of this study cohort, administrative health records will be used retrospectively to examine the developmental origins of health and disease [18], and prospectively to track and investigate the development of chronic disease in the future, starting at 5 years after the initial study is complete. Consent will be obtained to contact study participants for further clinical assessments, contingent on future funding.

Data from this study will provide an ideal opportunity for the exploration and potential discovery of new interactive mechanisms through which lifestyle factors affect health. We will be looking

to collaborate with other existing studies [19-21]with overlapping measures to replicate such findings, or increase sample size.. Findings from this research may be useful in guiding both clinical and health policy decisions, and will also facilitate the design and testing of personalized health promotion strategies. For example, if we are able to identify interactions between lifestyle factors and disease risk, such as a genetic variant that associates with short sleep to negatively impact health, a follow-up study could be designed looking to improve sleep hygiene specifically in the group with the risk variant.

Methods

Design

This is an exploratory observational cohort study with retrospective and prospective utilization of secondary data from administrative health records (**Figure 1**). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed where applicable in the development of this protocol manuscript [22].

Setting

Urban (Winnipeg) and rural (Morden, Winkler, Carman, Steinbach) areas with road access in southern Manitoba, Canada.

Objectives of the study

The objective of this study is to explore the complex interactions that exist between lifestyle, genetics, and gut microbiota, and how these relate to risk factors for chronic conditions, especially obesity, hypertension T2D, CVD, and CKD in Manitoba.

Inclusion and exclusion criteria

A sample of 800 Manitobans aged 30-46, stratified by sex, BMI, and geography **(Table 1)** are being recruited. Participants must have lived in Manitoba for a minimum of 5 years. Women who are pregnant or lactating are not eligible to participate. Additionally, because it is expected that very few of the 800 Manitobans who join TMPLR study from the general public will have reduced kidney function (eGFR <30 ml/min), 40 participants from Manitoba (20 female, 20 male, with no set stratification based on BMI or geography) who have severely reduced kidney function are being recruited from the renal health clinic at Seven Oaks General Hospital (SOGH), Winnipeg, MB. Therefore, the study has a recruitment goal of 840 participants.

Recruitment

Participants are recruited through the use of printed flyers, online advertisements purchased via Google, Facebook, and Twitter ad platforms and social media accounts, appearances in local TV, radio, and print media, and direct contact with community groups, such as churches, sports leagues, and community clubs. All patients who receive care in the SOGH renal health clinic, who are aged 30-46, have been living in Manitoba for a minimum of the last 5 years, and are able to provide informed consent are approached to enroll in the study as well.

Sample size

The sample size of TMPLR study was selected based on considerations of feasibility of recruitment, costs, and logistics. However, given established values from other sources [23] and our anticipated sample size of 840 participants, we estimate that we will have an 80% power (5% significance, 2-sided) to detect a minimum body fat difference of 2.5% for rare exposures (i.e.,

experienced by 10% of participants, such as smoking) and 1.7% for more common exposures (experienced by 25% of participants, such meeting the Canadian recommended 150 minutes of moderate-to-vigorous physical activity). Additional estimated minimum detectable differences are presented in **Table 2**. These lower limits should allow for the detection of clinically meaningful changes in these outcomes.

Data Collection and Assessments

On two consecutive days, participants come to either the urban TMPLR study site at the Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba or TMPLR's mobile research unit which travels to other areas of Winnipeg and southern Manitoba. TMPLR's mobile research unit is a custom built 12-meter mobile lab which is equipped with phlebotomy area, a dual-energy X-ray absorptiometer (DXA) and a bicycle ergometer with a metabolic cart. During this visit, participants complete questionnaires, undergo various health assessments, provide urine and fecal samples, and have fasting blood samples taken **(Figure 2, Table 3)**. The same protocols at were followed at both sites.

Questionnaires

Questionnaires capture socio-demographic characteristics, personal and family medical history, smoking (including electronic cigarette use), current diet (three Automated Self-Administered 24-h (ASA24) Dietary Assessment Tool recalls , Mindful Eating Questionnaire [24], Diet History Questionnaire [25] and The Three-Factor Eating Questionnaire [26]), alcohol consumption, physical activities, frailty using the Modified Fried Criteria [27], stress, sleep (Pittsburgh Sleep Quality Index [28]), cognition (Montreal Cognitive Assessment Questionnaire [29]), and childhood retrospective circumstances (adapted from the US Panel Study on Income Dynamics [30]).

Anthropometric assessment

Weight is measured after participants change into lightweight scrub tops and bottoms, with shoes removed, to the nearest 0.1 kg using a digital calibrated floor scale (7562EF, Taylor Precision Products, Oak Brook, IL, USA). Height is measured, without shoes, to the nearest 0.1 cm using a stadiometer (Model 206, SECA North America, Chino, CA, USA). BMI is calculated in kg/m^2. Waist circumference is measured in triplicate, to the nearest 0.1 cm at the umbilicus, between the last rib and the iliac crest using a fibreglass tape measure. Hip circumference is measured in triplicate at the widest portion of the buttocks and hips using a fibreglass tape measure. Body composition including fat mass, lean mass, percent body fat, visceral adipose tissue (VAT), and bone mineral density (BMD) are assessed using dual-energy X-ray absorptiometry (DXA, Lunar Prodigy Advance, GE Healthcare, Mississauga, ON, CAN) [31]. Scans are taken of the whole body, femoral neck, L1-L4 of the spine, and the non-dominant forearm.

Clinical health assessment

Participants' systolic and diastolic blood pressures are measured in triplicate, on the non-dominant arm in a sitting position using a validated oscillometric blood pressure monitor (BP760CAN, Omron, Burlington, ON, CAN). Participants are required to rest for 5-10 minutes before taking the measurement. Pulse wave velocity and augmentation index are measured on the non-dominant arm in a sitting position using a Mobil-O-Graph PWA Monitor and the HMS

Client Server Software (I.E.M Gmbh, Stolberg, Germany) according to the manufacturer's protocol on two consecutive days [32].

Collection of bio-specimens

Blood, urine, and fecal samples are obtained from study participants **(Supplementary protocols)**. Fasting blood samples are collected on two consecutive days via venipuncture by trained phlebotomists. Two blood samples on consecutive days are required to undertake the isotopic assessment of fractional cholesterol and triglyceride synthesis rates. Participants are asked to collect two urine samples at home; one sample is obtained prior to going to bed, and a second of the first morning void upon waking up. Participants also collect a fecal sample; they are provided a collection kit and instructed to collect a single sample from 3 separate places on the stool using a spoon attached to the cap of the collection tube. Participants are instructed to store the collected samples fecal in their household –20°C freezer with a provided ice pack, and urine samples in the fridge, until transport back to the study center, using provided ice pack for temperature control, where they are aliquoted and then stored at –80°C for future analysis [33].

Clinical chemistry in blood and urine

Clinical chemistry, including lipid profile, glucose, insulin, and renal and liver profiles will be measured via automated clinical chemistry analyzers (Cobas C111, C311 and e411, Roche Diagnostics Laval, QC). Blood and urine biomarkers such as leptin, glucagon, and melatonin will be measured via ligand binding assay (LBA) or enzyme-linked immunosorbent assay (ELISA). Red blood cell and plasma fatty acids will be measured by gas chromatography with flame ionization detections (GC-FID)[34]. Non-cholesterol sterols will be measured in plasma using GC-FID and mass spectrometry (MS) [35]. Vitamin C concentrations in the blood will be measure by high pressure liquid chromatography (HPLC) [36].

Microbiome analyses in fecal samples

Fecal samples will be subjected to genomic DNA extraction (Zymo Research, CA, USA) following the manufacturer's protocol. Experimental negative controls will be included in extraction protocols to confirm the reliability and consistency of the extracted nucleic acid. The V4 hypervariable region of 16S rRNA gene will be amplified, the sequencing library will be generated as described previously [37] and sequenced at the Gut Microbiome Laboratory, University of Manitoba. Samples will be multiplexed at the rate of 200 per run aiming for an average sequencing depth of 50,000 sequences per sample. The sequencing data will be deposited into the Sequence Read Archive (SRA) of NCBI (http://www.ncbi.nlm.nih.gov/sra) and accession numbers will be provided for future access.

Deuterium oxide administration

After the blood sample collection on day one, participants are given 0.7 grams of deuterium oxide per kilogram of estimated body water to drink. Body water is estimated as body weight (kg) x 0.60. This deuterium administration is used to enrich the body's water pool for the assessment of fractional cholesterol and triglyceride synthesis rates [38-40].

Physical activity and capacity testing

Physical activity level in TMPLR study participants is assessed using accelerometers (Actigraph GTX3bt, Penscola. FL, USA) worn for 1 week [41, 42]. Muscle strength is measured using a hand grip dynamometer. Cardiorespiratory fitness is assessed using a submaximal bike protocol which includes heart rate monitoring, and a metabolic cart (VMAX Encore, Carefusion, Unionville, Ont., Canada) to measure oxygen consumption and CO_2 output. Functional walking ability is assessed using a 5-meter gait speed test. Additionally, depressive symptoms, obesity history, frailty, low physical activity, and cognitive impairment are assessed by validated questionnaires [43-45].

Sleep assessment

Sleep in TMPLR study participants is measured objectively using accelerometers (Actigraph GTX3bt, Penscola. FL, USA [46]) worn for a week and subjectively by questionnaire (Pittsburgh Sleep Quality Index [28]). While there is a strong relationship between objective and subjective sleep reports, TMPLR study is collecting both because discrepancies may provide important clinical information reflecting early dysfunction [47, 48].

Dietary assessment

Study participants complete the Canadian version of the Diet History Questionnaire (DHQ) [25], which estimates the intake of common food items and includes portion size and dietary supplement questions. This questionnaire is on a TELEform for scanning data entry and creation of the data files. Participants also complete the Mindful Eating Questionnaire [24] to assess awareness of the physical and emotional sensations associated with eating, and The Three-Factor Eating Questionnaire [26] to assess dietary restraint, disinhibition and hunger in relation to eating. Participants also complete three dietary recall surveys using the Automated Self-Administered 24-hour Canada (ASA24®, NCI, Rockville, Maryland U.S. http://asa24.ca/)[49] dietary assessment tool, a web-based tool that enables multiple, automatically coded, self-administered 24-hour recalls. Participants enrolled from March 2016 to February 2017 used the ASA24-Canada-2014 edition; those enrolled after February 2017 used the ASA24-Canada-2016 edition. Both ASA24-Canada-2014 and ASA24-Canada-2016 use the same nutrient databases.

Early life experiences

Early-life exposures spanning the critical time windows of fetal development, birth, infancy and early childhood are documented in three ways: 1) through linkage with administrative health records (see Linkage to administrative health data), 2) by self-report, and 3) by maternal report. Administrative health data will provide method of birth, gestational age, birth weight, diagnosis codes for post-delivery hospitalization, and post-delivery drug prescriptions. Mothers of TMPLR study participants are asked to complete a TMPLR Mother's Questionnaire, adapted from the Nurses' Health Study [50], capturing key pregnancy, birth, and postpartum events such as method of birth; gestational age and birth weight; socioeconomic status at birth; maternal pre-pregnancy BMI and gestational weight gain; maternal smoking and diabetes during pregnancy; maternal prenatal care; breastfeeding initiation, exclusivity and duration; stressful life events during pregnancy and postpartum; and severe illness requiring hospitalization during infancy or early childhood. Early childhood socioeconomic status [51, 52] and stressful life events [53, 54] are also self-reported by TMPLR participants using the Childhood Retrospective Circumstances Questionnaire, adapted from the US Panel Study of Income Dynamics [30].

Data quality assurance and control

Methods of data collection (questionnaires, anthropometric assessment, and clinical health assessment) were standardized across the urban and mobile TMPLR study sites. Training of TMPLR study staff involved in data collection and data entry is regularly refreshed and all staff handling participant data are trained in compliance with the Manitoba Personal Health Information Act (PHIA). All data will be entered in the secure digital platform. A TMPLR study data model has been created to help in visualizing the different types of data the digital platform will contain. (**Supplementary figure 1)**

Linkage to administrative health data

At enrollment, TMPLR participants are asked to provide their PHIN and grant permission to link their study data with administrative health records (including hospital discharge abstracts, physician billing claims, and prescription records). These data are accessed through the Manitoba Centre for Health Policy (MCHP) Population Research Data Repository [55] and linkage is achieved using the PHIN, following the standard procedures established by the MCHP and the Manitoba Health Information Privacy Committee. The data linkage is used to capture retrospective information on early life as well as prospective information on numerous health outcomes, including diagnosis of hypertension, T2D, CVD, and CKD.

Statistical analyses

Statistical analyses will be undertaken in consultation with biostatisticians from the George and Fay Yee Centre for Healthcare Innovation (CHI) at the University of Manitoba. Lifestyle factors will primarily be used as explanatory variables, with chronic disease biomarkers or disease presence/absence as outcomes, in multivariable regression models. Moderating or mediating effects of genetics, gut microbiome, clinical characteristics, socio-economic status, and environmental factors will be explored. The potential confounding effects of health status and healthcare use on variable relationships will be examined using techniques such as propensity score or instrumental variable models [56-58].

Techniques appropriate for high-dimensional data will be adopted where needed. For example, clustering of lifestyle risk factors will be examined using latent variable modeling techniques (i.e., latent class analysis). Dimension reduction techniques for omics data, such as microbiome and genetic markers, will be applied [59].

The bioinformatics and statistical analyses of microbiome data will be performed as described previously [37] and will be updated based on recommendations and technology advancements between now and the point of processing of samples. Overall microbiota community structures, alpha diversity metrics, and relative abundances of operational taxonomic units (OTUs), will be tested for associations with lifestyle and health measures, with appropriate adjustment for multiple comparisons.

Non-response bias or inability to collect certain data, may affect the validity of analyses for survey data or biological measures, necessitating the use of multiple imputation methods if the pattern of missing data is deemed to be ignorable [60]. For non-ignorable missing data, selection and pattern mixture models will be examined in sensitivity analyses [61]. Due to the use of non-random sampling there is a risk of selection bias; survey weights and weighting of responses may be used to address this bias. Standardization or adjustment techniques may be used to address bio-specimen measurement error bias [62].

Specialized methodological investigations will be conducted for: 1) psychometric analyses of scales, including testing for differential item functioning and measurement invariance [63-65], 2) development of chronic disease risk prediction models [66, 67], 3) techniques to evaluate the quality of linked databases, including their accuracy, reliability, and completeness [68], 4) robust statistical methods for the analysis of outcome measures with non-normal (e.g. skewed) distributions [69, 70].

'Patient and Public Involvement Three focus group, one for healthcare providers and two for general public, and a public forum were held in the early design stages of this study to obtain input from Manitobans, on the study design and recruitment strategies. A study advisory board was also formed, and meets on a bi-annual basis. This advisory board includes healthcare providers, health researchers, and members of the public. The board provides input regarding study recruitment, progress and conduct, and will also provide input and suggestions regarding the dissemination of study results.

Provision of clinical results to participants

Individual results of the anthropomorphic measurements, blood pressure, pulse wave velocity, augmentation index, body composition, bone density, full lipid profile, fasting blood glucose, and renal and liver profile are to be provided to participants. Participants are referred to their primary care providers for further management if their results are beyond clinical reference ranges. Participants will not be provided their genetic and microbiome information.

Ethics and dissemination

Explicit informed consent is obtained from each individual prior to participation in the study. Eligible participants are verbally informed by trained research personnel regarding the nature and purpose of the study, given time to decide whether or not to participate, and have any questions or concerns answered prior to consent and at any point throughout the study. All participants are informed that they may withdraw from the study at any time without penalty and are remunerated for the portion of the study that they have completed up to that point. The full remuneration for study participation is $100 Canadian dollars, provided as cash or as a gift card.

Ethics approval has been obtained from the University of Manitoba Health Research Ethics Board prior to participant recruitment (protocol# HS18951). The study protocol has also been reviewed and approved by the Manitoba Health Information Privacy Committee in regards to the collection and use of PHIN, The St. Boniface Hospital Research Review Committee in regards to the processing of samples at the hospital, and the Winnipeg Regional Health Authority (WRHA) Research Access and Approval Committee (RAAC), the Southern Health Research Ethics Board, and the Interlake-Eastern Regional Health Authority Regional Ethics Committee, in regards to the study taking place in those health regions.

Data analysis, release of results, and publication of initial manuscripts are scheduled for 2019. Findings will be shared in peer-reviewed journals, and at regional, national, and international scientific conferences. Data and findings will also be presented to healthcare policymakers within Manitoba, to develop preventive strategies that reduce chronic conditions with the intention of reducing healthcare costs. Funding applications for future clinical follow in this study population have been submitted starting in 2017**.**

Discussion

TMPLR study has been uniquely designed to provide cross-sectional, retrospective and prospective observations that will improve our understanding of how lifestyle factors interact with each other and factors such as genetics and the gut microbiome to influence health and the risk of obesity, T2D, CVD, and CKD**.** The coordinated collection of lifestyle-gene-environment-microbiota-health data, including objective measurements such as DXA, activity monitoring, stable isotopic tracer methodologies, and direct measurement of physiological biomarkers; combined with the ability to retrospectively assess and prospectively follow health outcomes in participants using administrative health records, represents an unprecedented opportunity to collect data which can be used to improve chronic disease prevention and management.

Due to the voluntary non-random recruitment of participants, there may be an under-representation of those with lower health awareness, financial means, access, or time to participate. Attempts to counteract this are implicit in the stratified recruitment design. Comparisons between TMPLR study participants and general Manitoban population demographics may allow assessment of potential selection biases. A healthy volunteer effect may impact the ability to detect weak associations between lifestyle and disease risk, but this may attenuate with longer follow-up using administrative health data.

Given a projected sample size of 840 participants may be low for some of research questions that will be investigated, therefore harmonization and linking of data across multiple cohorts may be required. We will be looking to other studies which have undertaken overlapping measurements in order to increase sample sizes. The Canadian Longitudinal Study on Aging [19], the Toronto Nutrigenomics and Health [20], and The LifeLines DEEP [21] studies among others will be approached regarding the potential of data harmonization and cross-replication. TMPLR study will also be available to other researchers who are interested in collaboration or using the data for cross-replication.

In summary, TMPLR study will provide a unique platform of extensively phenotyped individuals that will be used to explore the interactions between lifestyle factors that associate with the development of, or protection from, obesity, hypertension, T2D, CVD, and CKD. The findings from this research platform will subsequently be used to develop and test preventive and restorative lifestyle and health strategies with the aim of improving the health and reducing healthcare costs at the individual and population levels.

Study status

Data collection started in March 2016. As of the August 15th 2018, data collection is ongoing and has passed 800 participants. Data collection is expected to end in December 2018.

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Author contributions

DSM and RCM developed the original concept of the study for the original grant application with input from co-investigators. DSM prepared the drafts of the study protocol manuscript and compiled feedback and changes from other authors. RCM and MG assisted in the preparation of the study protocol manuscript. PF developed the branding and logo for TMPLR study, and the manuscript figures and tables. NH prepared the data model and was involved in the public engagement. SB (project lead, indigenous health), HB (project lead, nutrition), JC, TAD (project lead, physical activity), PKE (project lead, genetics), EK (project lead, gut microbiome), LML (project lead, biostatistics), DEM (project lead, sleep), SBM, AR, NT, MBA (project lead, developmental origins of chronic disease), and PJJ (Director) are study co-investigators, and were all involved in writing the original grant application. All authors have carefully read, contributed to, and approved the final version of the study protocol manuscript.

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Competing interests statement

DSM, RCM, MG, SB, HB, JC, TAD, PKE, PF, NH, EK, LML, DEM, SBM, AR, NT, MBA, and PJJ have no competing interests to declare.

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Figure 1. The Manitoba Personalized Lifestyle Research Study overview

Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule

Table 1. The Manitoba Personalize Lifestyle Research Study recruitment targets by strata

Age	30-46 years N=800	
Sex	400 Males	400 Females

50% Male 50% Female								
Geography 72% Urban 28% Rural	288 Urban males		112 Rural males		288 Urban females		112 Rural females	
BMI 40% Normal (BMI <25) 60% Overweight (BMI ≥ 25)	116 Urban males BMI <25	172 Urban males BMI ≥ 25	45 Rural males BMI <25	67 Rural males BMI ≥ 25	116 Urban females BMI <25	172 Urban females BMI ≥ 25	45 Rural females BMI <25	67 Rural females BMI ≥ 25
+40 participants with severely reduced kidney function (eGFR <30 ml/min), 20 female, 20 male, with no set stratification based on BMI or geography								

Table 2. The Manitoba Personalized Lifestyle Research (TMPLR) study estimated minimum detectable differences

Variable	Mean or median used	Standard deviation used	Minimum difference at 10% exposure (percentage of mean)	Minimum difference at 25% exposure (percentage of mean)	References
Body fat (%)	41.3% Females 27.8% Males	7.7% 6.6%	2.5% (6.0)	1.7% (4.0)	[23]
Lumbar bone mineral density (BMD; g/cm^2)	1.042 Females 1.058 Males	0.121 0.127	0.041 (3.8)	0.028 (2.6)	[71]
Glomerular filtration rate (GRF; ml/min per 1.73 m^2)	107.6	16.8	5.4 (5.0)	3.8 (3.5)	[72]

Systolic blood pressure (mmHg)	116	12	6.5 (5.6)	4.5 (3.9)	[73]
Fasting Glucose (mmol/L)	4.94	0.61	0.20 (4.0)	0.14 (2.8)	[73]
Fasting insulin (µIU/mL)	7.83	7.50	2.40 (30)	1.67 (21%)	[74]
LDL cholesterol (mmol/L)	2.79	0.67	0.22 (7.8)	0.15 (5.4)	[73]
Waist circumference (cm)	80	10	3.2 (4)	2.2 (2.75)	[73]

Table 3. The Manitoba Personalized Lifestyle Research (TMPLR) study data, assessment tools and biological samples

Characteristic	***Data***	***Method, Instrument or Source***
Sociodemographic	Date of birth, Sex, Ethnicity, Marital status	TMPLR study questionnaire
Medical	Personal medical history, Family medical history, Medication(s), Pregnancy history	TMPLR study questionnaire, Administrative health records
	Cognition	Montreal Cognitive Assessment [29]
Lifestyle	Tobacco/smoking/vaping use, Alcohol use, Unintentional weight loss, Exhaustion, Depression	TMPLR study questionnaire
Physical activity	Frailty	Modified Fried Criteria [27]
	Physical activity	Paffenbarger physical activity index, Actigraphy [41, 42]
	Predicted VO2 max	Modified YMCA bike test with metabolic cart
Nutrition	Dietary patterns and habits	Mindful eating questionnaire[24], three-factor eating questionnaire [26], automated 24-hour dietary recall [49], Canadian dietary history questionnaire [25]
Early life	Childhood health, socio-demographic and socioeconomic status; Parental employment history	Childhood retrospective questionnaire, adapted from the US Panel Study on Income Dynamics[30]

	Maternal: pregnancy events, obstetrical history, infant feeding	TMPLR Mother's retrospective childhood questionnaire, adapted from the Nurses Health Study [50]
Socioeconomic	Employment, Home ownership, Education attainment, Income	TMPLR study questionnaire
Sleep and Stress	Duration of sleep	Actigraphy [46]
	Sleep Quality	Pittsburgh sleep quality index [28]
	Perception of stress, Daily life stressors	Community-based stress and coping survey
Anthropometric	Height	Wall-mounted stadiometer
	Weight	Digital scale
	Waist circumference, Hip circumference	Tape measure
	Body fat, Lean mass, bone mineral density	Dual energy X-ray absorptiometry [31]
Blood pressure	Systolic & diastolic	Automated sphygmomanometer
	Pulse wave velocity, Augmentation index	Mobil-O-Graph oscillometer [32]
Biomarkers	Blood clinical chemistry and biomarker assays	Fasting blood samples
	Urinary clinical chemistry and biomarker assays	Urine samples
	Microbiome 16S RNA sequencing	Fecal sample [37]

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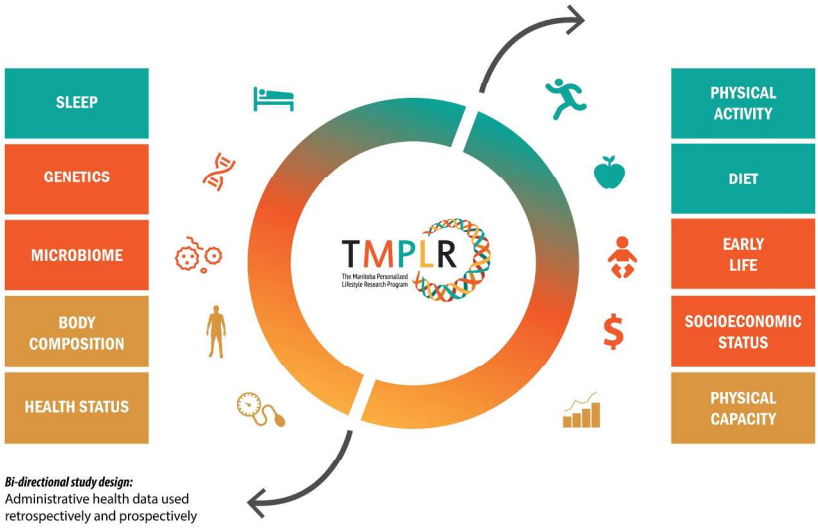

Figure 1. The Manitoba Personalized Lifestyle Research Study overview

230x130mm (300 x 300 DPI)

Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule

279x361mm (300 x 300 DPI)

TMPLR Data Model

Linking key: TMPLR ID #

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Urine Sample Collection Instructions

Please follow these instructions for urine collection. Research personnel will provide you with 2 urine collection cups labeled with time (night and day) and your TMPLR Study ID Number.

1. **Check your study ID on the collection tubes.** If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the samples.

2. Collect urine before going to bed tonight in the cup labeled "night". **Please write down the date and time of the sample was collected**. Store the sample in the fridge in the Ziploc bag provided.

3. Collect urine from the first time you pee after getting up in the morning, in the cup labeled "day". **Please write down the date and time of the sample was collected**. Store your samples in the fridge in the Ziploc bag provided.

4. Please bring the urine samples with you on your day 2 visit. TMPLR staff will collect the samples from you when you arrive.

If you have any questions, please contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.

Thank you for your cooperation!

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The Manitoba Personalized Lifestyle Research (TMPLR) Study

Stool Sample Collection Instructions

1. Freeze the ice packs provided by the study once you get home.

2. **Check your study ID** on the collection tubes (the two plastic tubes with blue cap). If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the sample.

3. Empty your bladder. Flush toilet. Place the collection unit under the rear part of the toilet seat with the round side pointing towards the back.

4. Have a bowel movement. **Collect 2 samples, one in each plastic tube, from 3 different places of the stool** using the spoon attached to the cap of the collection tubes. **Fill each sample tube about one third of the tube with stool sample**.

5. Close the tube tightly. Place each tube in a Ziploc bag provided. **Write down the date and time of the bowel movement** on the bag. Discard the used collection unit.

6. Wrap the collection tubes with the frozen ice packs, and keep them in the paper bag provided. Keep the collected sample in the freezer.

7. Return the stool samples wrapped with the frozen ice packs on day 2 of the measurements, or as soon as you can. TMPLR staff will collect the sample from you in the paper bag when you arrive.

If you have any questions, feel free to contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.

Collection Unit

Collection Tube with Spoon

Biospecimens collection

Blood samples will undergo analysis for numerous established and emerging health biomarkers, these include: total cholesterol, LDL-C, HDL-C, triglycerides, glucose, AST, ALT, insulin, glucagon-like peptide-1(GLP-1), leptin, c-reactive protein (CRP), fatty acids, HbA1c, T-regs, serum creatinine, blood urea nitrogen (BUN), non-cholesterol sterols, adipokines, cytokines,vitamin C, fat soluble vitamins, and lipidomic and metabolomics profiling. Gut microbiota analysis will be performed on stool samples. The assessment of gut microbiota is critical as increasing evidence suggests that some of the health effects of physical activity, sleep, and nutrition may be exerted through or modified via the gut microbiota. Participants' DNA will be obtained to determine genetic variations associated with chronic condition risk factors and telomere length measurement.

Urine Collection

Participants will be invited to collect urine from the time subsequent to going to bed (last void at bedtime not collected), to the first morning void. Urine samples will be received on day 2 (see Urine Sample Collection Instructions). Urine samples will undergo analysis for glucose, albumin, creatinine, melatonin, total protein and metabolomics profiling.

Blood collection

Fasting blood samples will be collected on both days (Day 1 and Day 2); they will be identified by participants' ID and separated as indicated (Table 5). Participants should come in fasting state (at least for 12h) and shouldn't take any alcoholic beverage for at least 48h before each visit. A total of 60 mL of blood will be obtained from participants (Appendix 21). Blood will be drawn by a certified phlebotomist and/or a register nurse.

Stool collection

Participants will be asked to collect stool sample from a bowel movement. After this, they will take samples randomly from 3 different places of the stool. Sample will be given to research personnel at the beginning of second appointment. Research personnel will provide instruction to volunteers at the end of the first visit (see Stool Sample Collection Instructions).

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Urine collection processing and collection instructions

Steps	Processing instructions
1	Receive urine sample and store it directly on 4˚C
2	Aliquot tubes should be labeled with participant ID
3	Number of labels required: 7 – 2.0 ml urine labels (if a urine sample was received)
4	If a urine sample is received proceed as follows: • Determine the volume of the urine • Pour some urine into a sterile container(to keep) • Aliquot urine into 2 -16 x 100 mm tubes and centrifuge
5	Aliquot as follows: 5 cryovials – 2.0 ml / vial (Seven Oaks) 2 cryovials – 2.0 ml / vial (McMillan)
6	Packaging of samples for transport: These samples must not thaw and must arrive frozen at the research lab Pack a transport box with ice packs and the frozen samples .Place the address label on the box. Ask the courier to return the transport box.

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Blood sample processing and collection instructions

Sample	Blood collection tube	Tube volume	Processing instructions	Aliquoting instructions	Analysis	Day
Serum	Red/grey SST tube	1 x 4mL	1. Invert 5 times 2. Room temp for 30 min 3. Spin for 10 min @ 1000 x *g*	1. Aliquot serum into cryovials[1] with brown[2] caps (0.5mL/tube) 2. Store at -80ºC	Insulin Lipid profile Glucose CRP GLP-1	1,2
Plasma	CPT tube (sodium heparin)	1 x 8 mL	1. Invert tube 8- 10 times 2. Spin for 30 min @1500- 1800 RCF 3. Resuspend by inverting 4. After addition of PBS spin for 15 min @ 300 RCF 5. Aspirate off as much supernatant without disturbing the pellet 6. Repeat wash in 10mL PBS 7. Resuspend pellet in 3mL freezing medium -10% DSMO (Sigma), 20% FCS (JRH Bioscience) in RPMI1640 (Gibco)	1. Aliquot entire contents above the gel and transfer to 15 mL Falcon tube 2. Add PBS (w/o Ca++ or Mg++) to make 15 mL 3. Store 1mL aliquots in -70°C using a Cyro-1°C/min freezing container.	T-Regulatory cells*	1
Plasma heparin	Green top (lithium heparin)	1x 4 mL	1. Invert 8 times 2. Spin immediately for 10 min @1300 x *g* 3.	1. Aliquot plasma into cryovials with green[3] caps (0.5mL/tube) 2. Store all fractions at -80ºC	C-reactive protein	1,2
RBC			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot RBC into cryovials with red[5] caps (0.5mL/tube) 2. Store all fractions at -80ºC	Fatty Acid Analysis	1,2
White blood cells Heparin			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot WBC (buffy coat) in 1 (one) Cryo.s™(RNase and DNase free	DNA extraction/ Telomere length	1,2

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Plasma EDTA	Purple top (K2 EDTA)	1X 10 mL	1.Invert 8 timesSpin immediate ly for 10 min@1300 x g 2.After addition of Methanol/ EDTA, spin @ 16,000g for 10 min. @1300 x g	1. Aliquot plasma into cryovials with yellow[5] caps (1.0 mL/tube) 2. Add to 1 plasma aliquot (0.5Ml), 1 volume of sample to 4 volumes of 90% methanol/water/1 mM EDTA 3. Place on dry ice for 5 min 4. Store all fractions at -80ºC	Ascorbic acid	1,2
Plasma EDTA			1.Invert 8 times 2.Spin immediately for 10 min @ 1300 x g	1.Aliquot plasma in cryovials with purple[6] caps (0.5ml/tube) 2.Store all fractions at -80ºC	Leptin Glucagon Oxidized phospholipids and oxylipins	1,2
Plasma EDTA			1.Invert 8 times 2.Spin immediately for 10 min @ 1300 x g	1.Aliquot RBC into cryovials with purple[5] caps (0.5mL/tube) 2.Store all fractions at -80ºC	Non-cholesterol sterols	1,2
White blood cells EDTA			1.Invert 8 times 2.Spin immediately for 10 min @ 1300 x g	Aliquot WBC (buffy coat) in 1 (one) Cryo.s™(RNase and DNase free vials)[4] 2.Store at -80ºC	DNA extraction/ Telomere length	1,2